



The Auk 123(3):775–783, 2006
© The American Ornithologists' Union, 2006.
Printed in USA.

TELOMERE SHORTENING IN A LONG-LIVED MARINE BIRD: CROSS-SECTIONAL ANALYSIS AND TEST OF AN AGING TOOL

FRANS A. JUOLA,^{1,3} MARK F. HAUSSMANN,² DONALD C. DEARBORN,¹
AND CAROL M. VLECK²

¹Department of Biology and Program in Animal Behavior, Bucknell University, Moore Avenue, Lewisburg,
Pennsylvania 17837, USA; and

²Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, Iowa 50014, USA

ABSTRACT.—A correlation between length of telomere restriction fragments (TRFs) and age has recently been demonstrated in several bird species. Comparisons of different-aged individuals within a population have shown that TRFs typically shorten with age and that this shortening continues throughout the life span of the species. In addition, it has been shown that telomere rate-of-change (TROC) correlates tightly with life span across several bird species. Previous studies of long-lived birds, however, have shown exceptions to these trends, demonstrating no declines in TRF length in adults in some cases and increases in TRF length with age in other cases. Here, we report known ages of individuals from a colony of Great Frigatebirds (*Fregata minor*) based on recaptures of leg-banded birds, including two individuals that were at least 44 years of age, the oldest Great Frigatebirds ever reported. Using a previously developed molecular technique, we report a predictable, nonlinear decline of TRF length with age in this population. Telomere restriction fragments decline more rapidly early in life but continue to shorten throughout the life span examined. The rate of decline in TRF for this species does not fit the tight correlation previously reported between TROC and life span in other species. Finally, we tested the ability to estimate age and age structure of breeding females on the basis of the calibration of TRF length and individuals of known age. Because of the slow telomere-attrition rate and the variability observed in TRF lengths at given ages, estimations of age of individuals and of estimated age structure of breeding birds in this population are not particularly reliable. Received 29 May 2004, accepted 3 October 2005.

Key words: age structure, aging, *Fregata minor*, Great Frigatebird, life span, telomere.

Encogimiento del Telómero en un Ave Marina Longeva: Análisis de Secciones Cruzadas y Evaluación de una Herramienta de Envejecimiento

RESUMEN.—Recientemente se ha demostrado una correlación entre el largo de los fragmentos de restricción de los telómeros (FRT) y la edad en varias especies de aves. Las comparaciones de individuos de edades diferentes de una población han mostrado que los FRT típicamente se acortan con la edad y que este encogimiento continúa a lo largo de la vida de estas especies. De modo adicional, se ha demostrado que la tasa de cambio del telómero (TCT) se correlaciona fuertemente con la longevidad en varias especies de aves. Sin embargo, estudios previos de aves

³Present address: Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, Florida 33124, USA. E-mail: frans@bio.miami.edu

longevas han mostrado excepciones a estas tendencias, demostrando la falta de disminuciones en el largo de los FRT de los adultos en algunos casos e incrementos en el largo de los FRT con la edad en otros casos. Aquí, documentamos datos sobre edades conocidas de individuos de una colonia de *Fregata minor* basados en recapturas de aves anilladas en las piernas, incluyendo dos individuos de por lo menos 44 años de edad, el individuo conocido más viejo de *F. minor*. Usando una técnica molecular previamente desarrollada, reportamos una disminución no lineal predecible del largo de los FRT con la edad en esta población. Los FRT disminuyeron más rápidamente temprano en la vida pero continuaron acortándose a lo largo del período de vida examinado. La tasa de disminución en los FRT para esta especie no concuerda con la estrecha correlación reportada anteriormente entre la TCT y el período de vida de otras especies. Finalmente, evaluamos la capacidad de estimar la edad y la estructura de edades de las hembras reproductivas basándose en la calibración del largo de los FRT y en individuos de edad conocida. Debido a la lenta tasa de desgaste del telómero y a la variabilidad observada en los largos de los FRT a determinadas edades, las estimaciones de la edad de los individuos y de la estructura de edades estimada a partir de las aves reproductivas en esta población no son particularmente confiables.

TELOMERES ARE PROTEIN–DNA complexes that cap the ends of linear eukaryotic chromosomes (Blackburn 1991, Zakian 1995). They serve a variety of functions, including providing chromosomal stability (Watson 1972, Prowse and Greider 1995), aiding in the replication process (Meyne et al. 1989), inhibiting rearrangements and fusions of broken chromosomes (McClintock 1941, Smogorzewska et al. 2002), and aiding in chromosome segregation (Kirk et al. 1997, Hande et al. 1999). The highly conserved repeated telomere sequence TTAGGG is present in at least 91 vertebrate species tested from a variety of classes, including bony fishes, reptiles, amphibians, birds, and mammals (Meyne et al. 1989). By nature of the linear DNA replication process, DNA polymerase is unable to complete the replication of the 3' end of DNA strands. Therefore, during each replication cycle of a cell, some telomeric repeats are left as single-stranded ends that are eventually lost to breakage or degradation by exonucleases (Watson 1972). Cultured human cells have a finite proliferative capacity, reaching a replication limit after a certain number of cell divisions (Hayflick and Moorhead 1961, Vaziri et al. 1994, Aikata et al. 2000). This limit is correlated with a critical telomere-length threshold. Therefore, cellular senescence may be regulated by a molecular clock, specifically, the gradual decrease in telomeres that occurs during each cellular replication (Olovnikov 1973). In rare cases, telomere

length may be maintained or even lengthened through the activity of the ribonucleoprotein telomerase (Greider and Blackburn 1985).

Telomere restriction fragments (TRFs) are isolated using a restriction enzyme that cleaves DNA at specific restriction sites. Using an enzyme that cleaves at a restriction site common within the DNA but that does not occur within the telomeric region allows for separation of the telomere fragment from the remaining DNA with minimal nontelomeric DNA attached. Recent work has shown that TRFs shorten predictably with age in at least four avian species—Adélie Penguin (*Pygoscelis adeliae*), Common Tern (*Sterna hirundo*), Tree Swallow (*Tachycineta bicolor*), and Zebra Finch (*Taeniopygia guttata*)—whereas in a fifth species, Leach's Storm-Petrel (*Oceanodroma leucorhoa*), TRF length increases with age (Hausmann and Vleck 2002, Hausmann et al. 2003). Whether increasing or decreasing, TRF length continued to change across the life span of each of these species. In addition, a strong correlation between telomere rate of change (TROC) and maximum life span was observed across the five species, including the longest-lived of the group, Leach's Storm-Petrel (maximum observed life span of 36 years; Hausmann et al. 2003), which exhibited an increase in TROC with age (Hausmann et al. 2003, Vleck et al. 2003).

Telomeres in two additional long-lived species, the European Shag (*Phalacrocorax aristotelis*)

and Wandering Albatross (*Diomedea exulans*), did not exhibit linear patterns of increase or decline throughout their life span (Hall et al. 2004). For both species, telomere lengths decreased between the chick stage and adulthood, but showed no further change among adults.

Clarifying the relationship between life span, telomere length, and TROC may contribute to our understanding of life spans and longevity in birds. Long-lived birds, in particular, represent intriguing possibilities for further study in this regard. In addition, the relationship between telomere length and age presents the possibility that telomeres could be used as a tool for aging individual birds or for estimating the age structure of a given population. If telomere length and age of some individuals of a given population are known, a calibration of this relationship can be used to age additional individuals from the population whose histories are not known.

Here, we explore the relationship between TRF length and age in a free-living, Hawaiian population of Great Frigatebirds (*Fregata minor*). The life span of Great Frigatebirds is unknown. However, the life-history traits exhibited by this species are extreme, even among marine birds, which suggests a long life span (Metz and Schreiber 2002). Seabird biologists have speculated that they typically live from 25 to 30 years and perhaps for a maximum of 40 years, though very little data support these assertions. To date, the oldest reported age for a Great Frigatebird is 37 years (Metz and Schreiber 2002).

There were three goals of our investigation: (1) to report known ages from a colony of Great Frigatebirds based on the recapture of banded birds; (2) to define the relationship between TRF length and age across the life span, including nestlings and adults, based on known-age birds; and (3) to assess the usefulness of TRF lengths for estimating individual age and population age structure in breeding female Great Frigatebirds.

METHODS

Colony history.—The study colony was located on Tern Island, in the Northwest Hawaiian Islands (23°45'N, 166°17'W). Tern Island is one of 10 small islands in the French Frigate Shoals atoll. The island is administered by the U.S. Fish and Wildlife Service (USFWS). It encompasses

~14 ha and is a breeding area for ~4,000 Great Frigatebirds each year (Dearborn and Anders 2006). Great Frigatebirds and other seabirds were banded in the Hawaiian Islands from 1963 to 1969 by the Pacific Ocean Biological Survey Program operated by the Smithsonian Institution. Banding of Frigatebirds was resumed on Tern Island from 1987 to 1991 by the USFWS. Banded birds in this colony were too few to assess age structure accurately but were sufficient to calibrate the relationship of TRF length and age.

Blood sample collection.—We used known-age individuals from the Tern Island colony of Great Frigatebirds to determine the relationships between TRF length and age. In August 2002, the colony was checked daily for presence of banded birds, for which banding history would be available. Banded birds were captured by hand, and 90 μ L of whole blood were collected from the femoral artery. Nestlings were also sampled, systematically around the colony, to extend the age range of known-age birds. All blood samples were immediately mixed into cold acid citrate dextrose buffer (ACDB). A second sample was collected from nestlings and mixed in 2% EDTA (approximately one part blood:four parts buffer) for later sex determination. All samples were stored at 4°C for 1–3 weeks until they could be transported to the lab. In the lab, the ACDB samples were microcentrifuged at 3,000 rpm for 1 min, washed with phosphate buffer solution three times, resuspended in glycerol storage buffer to prevent lysis, snap-frozen in liquid nitrogen, and stored at –80°C. Later, banding records were obtained from the USFWS files to determine the ages of the banded adult birds sampled in 2002. In cases in which birds had been banded as nestlings, the exact age was determined. In other cases, birds were originally banded as breeding adults and, thus, the exact age could not be determined. For these birds, seven years were added to the number of years between the original banding year and our recapture in 2002, because this is believed to be the minimum age of first breeding in Great Frigatebirds (Metz and Schreiber 2002). In 2003, 82 successfully breeding females (i.e., those brooding a nestling recently hatched) were selected at random, and ~90 μ L of blood were collected as described above.

DNA extraction and digestion.—DNA from nestling samples collected in EDTA was extracted

using an alcohol-based DNA precipitation technique (H. L. Gibbs unpubl. protocol). We determined the sex of all nestlings by the standard molecular technique using the P2/P8 primers from Griffiths et al. (1998) and amplifying a region of the CHD-1 gene on the avian sex chromosomes. All adults were sexed by plumage.

The remaining samples previously frozen in glycerol storage buffer were thawed for 10 min and then microcentrifuged for 5 min at 10,000 rpm to remove the glycerol storage buffer. Cells were washed three times with phosphate buffer solution (PBS), each wash followed by microcentrifugation and buffer removal. DNA from 5 μ L of packed erythrocytes was extracted into agarose plugs to provide intact chromosome-sized DNA according to the manufacturer's specifications (Biorad, Hercules, California). Agarose plugs (one plug per individual sample) were restriction digested with *Hinf* I (80 U; New England Biolabs, Beverly, Massachusetts) to cut the DNA into short fragments. Restriction enzymes cleave DNA at specific restriction sites, and because the *Hinf* I restriction site is common but does not occur within the telomeric repeat sequence, digestion yielded relatively long TRFs that contain the telomere attached to a portion of DNA in the subtelomeric region. Restriction digest was at 37°C for 4 h. Upon completion, more *Hinf* I (70 U) was added, and plugs were further digested at 37°C for an additional 2 h to ensure that digestion was complete.

Gel electrophoresis and hybridization.—Agarose plugs were sliced into halves, and one-half of each plug (~10 μ g DNA) was separated on a 0.6% nondenaturing agarose gel (2.8 V cm^{-1}) for 21 h. The gel was kept at 15°C, and the 1 \times tris acetate EDTA (TAE) buffer was circulated for the duration of the separation because TAE, which has a low buffering capacity, is easily depleted during long runs. On each gel, three ³²P-labeled lambda ladders were run in different lanes to test for uniformity of DNA migration across the gel. The between- and within-gel coefficients of variation were determined by running one sample three times on each gel. The intra- and inter-gel coefficients of variation were 4.8% and 5.4%, respectively. Upon completion of the run, the gel was soaked in 2 \times salt sodium citrate (SSC) for 30 min at room temperature to control stringency during the subsequent hybridization step. The gel was then dried under vacuum and was prehybridized for 1 h

at 37°C in Church buffer followed by hybridization overnight at 37°C with a ³²P-labeled (CCCTAA)₄ oligonucleotide probe (~90,000 cpm mL⁻¹) in Church buffer (Sambrook et al. 1989). Each telomere has a single-stranded overhang to which the radiolabeled probe binds, providing a measurable signal for telomere measurement. After hybridization, gels were washed with 0.25 \times SSC for 30 min at room temperature, followed by two 1-h 0.25 \times SSC washed at 37°C to remove any unbound probe.

Telomere restriction fragment length analysis.—Circulating erythrocytes are descendants from bone-marrow stem cells of different replicative history, so the TRF lengths of erythrocytes measured in a sample of erythrocytes will be variable. Because the DNA sequence is different from each chromosome within a cell, *Hinf* I cuts at variable distances from the telomere, also introducing variability into our measurement of TRF length. For these reasons, the resulting fragments from each chromosome and from each cell vary in size, producing a smear on the gel rather than a sharp band. We used a phosphor imager system (Molecular Dynamics, Sunnyvale, California) to visualize this population of TRFs and densitometry using TELOMETRIC, version 1.2 (Grant et al. 2001) to determine the position and strength of the radioactive signal in each gel lane over the range from the bottom of the TRF smear to just below the limit of mobility, the migration limit for the longest telomere fragments. The mean TRF length is determined by calculating the average fragment length for each gel lane using the equation

$$\text{length} = \Sigma (\text{OD}_i \times L_i) / \Sigma (\text{OD}_i)$$

where OD_i is the densitometry output at position i (arbitrary units), and L_i is the length of the DNA in base pairs at position i .

After calculating TRF lengths for each Great Frigatebird of known age in 2002, we used a regression analysis with age as the independent variable and TRF length as the dependent variable to calibrate the relationship between these variables. We square-root-transformed age before performing this analysis to improve the fit of the regression, because the relationship between the untransformed variables was nonlinear. We then predicted age with a new set of TRF lengths from birds of unknown age by algebraic rearrangement of the linear regression

equation (calculated in square-root form first, then back-transformed to years by squaring). We then used the technique of inverse prediction (Zar 1996) to calculate upper and lower 95% confidence limits (CLs) for each age estimate (each data point around the regression line) on breeding females in 2003.

RESULTS

In 2002, we estimated that <1% of all Great Frigatebirds in our study colony on Tern Island were banded. Thirty-five previously banded adults were recaptured. The ages of these birds fell into two categories, corresponding to the two periods of Great Frigatebird banding at French Frigate Shoals. Twenty birds had known ages from 9 to 15 years, and 15 had known ages from 35 to 44 years. Three of the oldest birds were originally banded as breeding adults. They were recaptured 36 years later in one case and 37 years later in two cases. Assuming that the minimum breeding age is 7 years, 7 years was added to the time between captures, resulting in minimum age estimates of 43 and 44 years for these individuals.

For the analysis of TRF length versus age, we had available blood samples for 12 nestlings and 27 previously banded adults from 2002. We excluded the three birds for which age was a minimum estimate. All nestlings sampled were estimated to be between two and four months of age based on size and developmental characteristics.

Mean TRF length decreased significantly with age for these 36 individuals ($F = 98.04$, $df = 1$ and 34 , $P < 0.001$, $r^2 = 0.74$, slope = -36.1 ± 3.7 (mean \pm SE); Fig. 1A). Similarly, the linear regression using square-root-transformed age showed a significant decline in TRF length with age ($F = 150.38$, $df = 1$ and 34 , $P < 0.001$, $r^2 = 0.82$, slope = -256.7 ± 20.9 ; Fig. 1B). The regression equation predicting TRF length (TRF = $-256.71 \times \sqrt{\text{Age}} + 9675.41$) is rearranged to predicted age from TRF lengths: age (years) = $[(\text{TRF} - 9675.41) / -256.71]^2$. Using square-root-transformed age, the linear regression equation predicts that TRF lengths decrease by ~ 257 base pairs (bp) per year in the first year of life. The rate of shortening slows to ~ 120 bp per year in 39-year-old birds. The relationship between TRF length and age did not differ for males and females in terms of slope (analysis of covariance [ANCOVA], $F =$

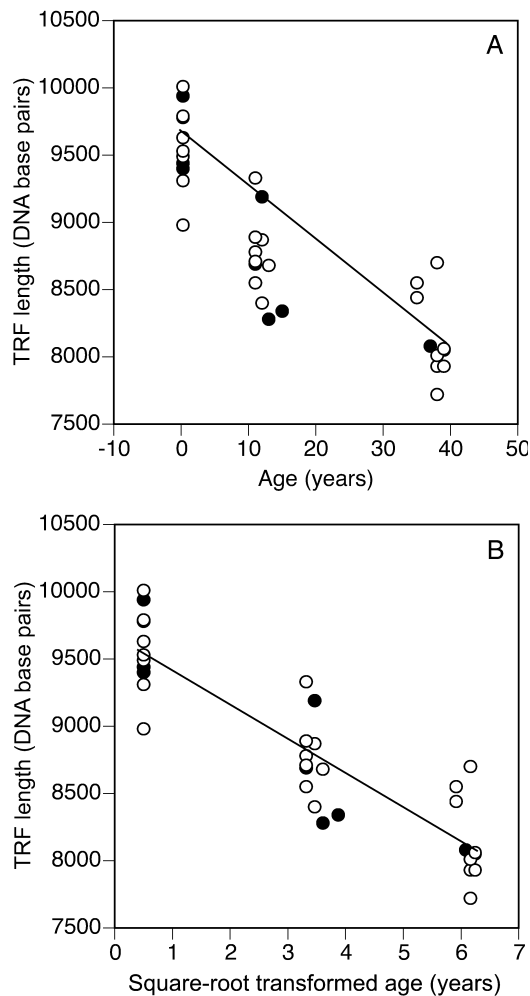


FIG. 1. (A) Regression of telomere restriction fragment (TRF) length and known age in Great Frigatebirds, Tern Island, French Frigate Shoals, 2002. Open circles represent females; dark circles represent males. (B) Regression of telomere restriction fragment (TRF) length and square-root-transformed age in Great Frigatebirds, Tern Island, French Frigate Shoals, 2002. Open circles represent females; dark circles represent males.

1.59 , $df = 1$ and 32 , $P = 0.216$) or y -intercept (ANCOVA, $F = 0.23$, $df = 1$ and 33 , $P = 0.635$). When nestlings were removed from the analysis, TRF length still declined significantly with untransformed age in adult Great Frigatebirds ($F = 28.54$, $df = 1$ and 22 , $P < 0.001$, $r^2 = 0.57$, slope = -24.1 ± 4.5).

Blood samples were collected in 2003 from 102 females that were brooding nestlings. Eighty-one of these samples were used in TRF analysis. Using the regression equation established from known-age birds from 2002, age estimates along with 95% confidence limits (CLs) were calculated for the other 81 individuals. Age estimates ranged from 1 to 124 years, with a mean of 31.6 ± 3.6 years (SE; Fig. 2). The range of the 95% CLs for each age estimate at a particular TRF was wide and increased as the estimated age increased.

DISCUSSION

Ten of the 35 Great Frigatebirds recaptured in 2002 were >37 years of age; these are the oldest Great Frigatebirds ever reported. Furthermore, three of these individuals were originally banded as adults; hence, their ages (43 in one case, 44 in two cases) represent minimum estimates, with their actual ages possibly being substantially greater. We conclude that the maximum life span of Great Frigatebirds in the wild is at least 44 years. Mean mass of Great Frigatebirds in our study was 1,400 g. Differences in body size can explain some of the variations observed in

life span, because larger animals typically live longer than smaller ones (Homes and Austad 1995). The equation predicting life span in birds based on mass is $\text{life span} = 17.6 \times (\text{mass in kg})^{0.20}$ (Lindstedt and Calder 1976). A maximum life span of 44 years for Great Frigatebirds represents a 234% increase from the predicted maximum of 18.8 years. Great Frigatebirds, therefore, are extremely long-lived given their body size, particularly when considering that this percentage is a minimum estimate.

As in several other bird species examined previously (Hausmann et al. 2003), TRF lengths declined predictably with age in Great Frigatebirds. The other species showing declines, however, all had substantially shorter maximum life spans than Great Frigatebirds. The other species in which the relationship between TRF length and age has been examined, and which most closely approach Great Frigatebirds in life span, are the Wandering Albatross and Leach's Storm-Petrel. The maximum observed life span of the Leach's Storm-Petrel is 36 years (Hausmann et al. 2003). This represents a 380% increase from the expected life span of 9.5 years, given the reported mass of 45 g for Leach's Storm-Petrels (Hausmann et al. 2003). In contrast with Great Frigatebirds, TRF length increases with age in Leach's Storm-Petrels and maintains a relatively constant length (among adults) in Wandering Albatrosses. Hausmann et al. (2003) suggest that telomere lengthening in Leach's Storm-Petrels is an adaptation to allow a longer life span; although this may be true, our results with Great Frigatebirds show that a long life span is possible in birds without this adaptation.

The rate of decline in TRF length slowed with age in our population. This pattern is found in a number of mammals, including humans (Frenck et al. 1998, Rufer et al. 1999, Zeichner et al. 1999, Friedrich et al. 2001, Brümmendorf et al. 2002), and has also been reported previously in two species of birds, the Wandering Albatross and European Shag (Hall et al. 2004). In both the latter two species, telomeres showed no decline among adults. This contrasts with our findings, because TRF lengths continued to shorten across the adult life span sampled in our study, albeit at a slower rate than during early life. Faster declines in telomere lengths early in life can be explained, at least in part, by faster rates of cell growth and cell division (Frenck et al. 1998, Rufer et al. 1999).

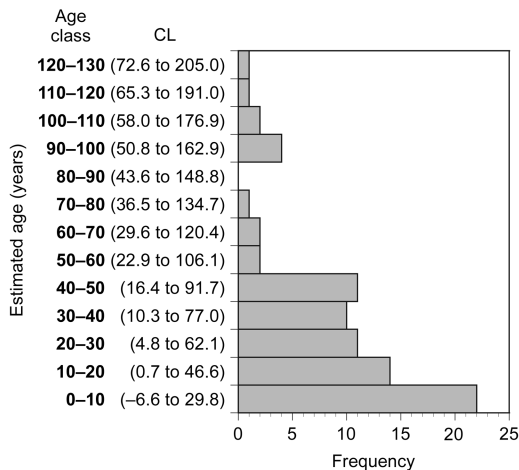


FIG. 2. Age structure determined from estimated ages on the basis of telomere restriction fragment lengths of Great Frigatebird females brooding nestlings in 2003, Tern Island, French Frigate Shoals. Confidence limits (CLs) listed represent the lower CL for the youngest age and upper CL for the oldest age found in each 10-year age class.

The relationship between TRF length and age at ages >39 years remains unknown for Great Frigatebirds. In calculating estimated ages of successfully breeding females, we make the assumption that TRF lengths continue to decline in a similar fashion after 39 years of age. If that is the case, our data suggest that Great Frigatebirds in this population live much longer than the oldest known bird according to our band returns (≥ 44 years). The lower 95% CL on the oldest age estimate was 75 years.

The TROC for Great Frigatebirds was negative, ranging from a decline of 257 to 120 bp per year across the known-age life span (0–39 years of age). The relationship between TROC and life span reported for other bird species (Hausmann et al. 2003) would predict a positive TROC (TRF length increase with age) of ≤ 250 bp per year for Great Frigatebirds, even with the modest maximum life-span estimate of 44 years. Such an increase could perhaps be obtained through activation of telomerase. The negative TROC reported here provides further evidence that the true relationship between TROC and age in birds is nonlinear (Hall et al. 2004) and similar to that reported for mammals (Hausmann et al. 2003).

Cross-sectional analyses such as this one may not precisely mirror telomere changes occurring in individuals over their life span (Arking 1998). Selective mortality in individuals with rapid telomere loss could result in cross-sectional data biased toward individuals with slow telomere loss (Hall et al. 2004). Longitudinal data are difficult to gather for obvious reasons, particularly for long-lived species such as marine birds, where decades would be needed to obtain a sufficient time span for a meaningful telomere comparison across an individual's lifetime. There has been only one study tracking individual telomere change over time in birds (Hall et al. 2004). The European Shag showed high inter-individual variation in telomere loss over time from nestlings to adults ranging from two to six years of age. Much of this inter-individual variation was explained by environmental factors, such as hatching date and chick mass.

Telomere restriction fragment length produces imprecise age estimates, with wide confidence limits for each estimate. The relatively slow decrease of TRF length with age, coupled with the variation observed in individuals at or near the same age, made predicting age in

this population difficult. Additional sampling from this population of individuals of known age, especially those older than 39 years and within the age gaps that currently exist in the banding data, might allow some narrowing of the confidence limits. Some of the variation in TRF length is undoubtedly attributable to actual individual differences in initial lengths and rates of shortening, but some may also be attributable to error associated with the measurement technique. Improved techniques for measuring telomere lengths may eliminate more of this variation as well, and in so doing improve the ability to estimate age (Nakagawa et al. 2004). The most accurate age estimates based on telomere lengths should come from species with large changes in telomere length over their life span, along with little variation in initial telomere length (Nakagawa et al. 2004). Neither of these conditions held for this population of Great Frigatebirds.

Although samples were collected at random from successful breeding females throughout the entire breeding season, the age estimates we use to construct an age distribution undoubtedly contain error. Therefore, the age structure we estimated on the basis of these ages is also subject to error. The age structure is averaged over many individuals; thus, the importance of error should be reduced, giving more confidence to age-structure data than to individual age estimates. However, because additional error is potentially introduced by extrapolating age estimates from TRF lengths beyond the range of known-age individuals, the true accuracy of the age structure estimate in this case is unknown. The odd feature of the estimated age structure is the long tail at great ages. Testing this feature will require many decades of additional observation of banded individuals.

Another consideration in using telomere lengths to predict age is telomere damage associated with oxidative stress. Internal metabolic processes, as well as external factors such as poor nutrition, can cause oxidative damage (Goyns 2002), which may accelerate telomere shortening (von Zglinicki et al. 2000). Telomeres may, therefore, reflect a composite picture of chronological age combined with the amount of physiological stress an individual has experienced over its lifetime. Such a measure has been termed "biological age" (Harley et al. 1992, Aviv 2002). Biological age may be more important

than chronological age, because it may be a better indicator of future health and longevity (Cawthon et al. 2003). The eight individuals observed in the long right tail of the estimated age distribution (Fig. 2), all of which have age estimates of >72 years, may be considered very old in a biological sense, regardless of their true chronological age.

ACKNOWLEDGMENTS

Funding sources for this project included an AOU student research award, a Sigma Xi grant-in-aid of research, and Bucknell's Department of Biology and Program in Animal Behavior. The U.S. Fish and Wildlife Service provided logistic support. B. Flint, D. Horvath, and the Tern Island managers greatly facilitated this support. Thanks also to the enthusiastic volunteers on Tern Island who assisted with field work. Three reviewers made helpful suggestions about the manuscript.

LITERATURE CITED

- AIKATA, H., H. TAKAISHI, Y. KAWAKAMI, S. TAKAHASHI, M. KITAMOTO, T. NAKANISHI, Y. NAKAMURA, F. SHIMAMOTO, G. KAJIYAMA, AND T. IDE. 2000. Telomere reduction in human liver tissues with age and chronic inflammation. *Experimental Cell Research* 256:578–582.
- ARKING, R. 1998. *Biology of Aging: Observation and Principles*, 2nd ed. Sinauer Associates, Sunderland, Massachusetts.
- AVIV, A. 2002. Chronology versus biology: Telomeres, essential hypertension, and vascular aging. *Hypertension* 40:229–232.
- BLACKBURN, E. H. 1991. Structure and function of telomeres. *Nature* 350:569–573.
- BRÜMMENDORF, T. H., J. MAK, K. M. SABO, G. M. BAERLOCHER, K. DIETZ, J. L. ABKOWITZ, AND P. M. LANSDORP. 2002. Longitudinal studies of telomere length in feline blood cells: Implications for hematopoietic stem cell turnover *in vivo*. *Experimental Hematology* 30:1147–1152.
- CAWTHON, R. M., K. R. SMITH, E. O'BRIEN, A. SIVATCHENKO, AND R. A. KERBER. 2003. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 361:393–395.
- DEARBORN, D. C., AND A. D. ANDERS. 2006. Demography and reproductive ecology of Great Frigatebirds. *Atoll Research Bulletin* 543: in press.
- FRIEDRICH, U., M. SCHWAB, E. U. GRIESE, P. FRITZ, AND U. KLOTZ. 2001. Telomeres in neonates: New insights in fetal hematopoiesis. *Pediatric Research* 49:252–256.
- FRENCK, R. W., JR., E. H. BLACKBURN, AND K. M. SHANNON. 1998. The rate of telomere sequence loss in human leukocytes varies with age. *Proceedings of the National Academy of Sciences USA* 95:5607–5610.
- GOYNS, M. H. 2002. Genes, telomeres and mammalian ageing. *Mechanisms of Ageing and Development* 123:791–799.
- GRANT, J. D., D. BROCCOLI, M. MUQUIT, F. J. MANION, J. TISDALL, AND M. F. OCHS. 2001. TELOMETRIC: A tool providing simplified, reproducible measurements of telomeric DNA from constant field agarose gels. *Biotechniques* 31:1314–1316.
- GREIDER, C. W., AND E. H. BLACKBURN. 1985. Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. *Cell* 43:405–413.
- GRIFFITHS, R., M. C. DOUBLE, K. ORR, AND R. J. G. DAWSON. 1998. A DNA test to sex most birds. *Molecular Ecology* 7:1071–1075.
- HALL, M. E., L. NASIR, F. DAUNT, E. A. GAULT, J. P. CROXALL, S. WANLESS, AND P. MONAGHAN. 2004. Telomere loss in relation to age and early environment in long-lived birds. *Proceedings of the Royal Society of London, Series B* 271:1571–1576.
- HANDE, M. P., E. SAMPER, P. LANSDORP, AND M. A. BLASCO. 1999. Telomere length dynamics and chromosomal instability in cells derived from telomerase null mice. *Journal of Cell Biology* 144:589–601.
- HARLEY, C. B., H. VAZIRI, C. M. COUNTER, AND R. C. ALLSOPP. 1992. The telomere hypothesis of cellular aging. *Experimental Gerontology* 27:375–382.
- HAUSSMANN, M. F., AND C. M. VLECK. 2002. Telomere length provides a new technique for aging animals. *Oecologia* 130:325–328.
- HAUSSMANN, M. F., D. W. WINKLER, K. M. O'REILLY, C. E. HUNTINGTON, I. C. T. NISBET, AND C. M. VLECK. 2003. Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. *Proceedings of the Royal Society of London, Series B* 270:1387–1392.
- HAYFLICK, L., AND P. S. MOORHEAD. 1961. The serial cultivation of human diploid cell

- strains. *Experimental Cell Research* 25: 585–621.
- HOMES, D. J., AND S. N. AUSTAD. 1995. The evolution of avian senescence patterns: Implications for understanding primary aging processes. *American Zoologist* 35: 307–317.
- KIRK, K. E., B. P. HARMON, I. K. REICHARDT, J. W. SEDAT, AND E. H. BLACKBURN. 1997. Block in anaphase chromosome separation caused by a telomerase template mutation. *Science* 275:1478–1481.
- LINDSTEDT, S. L., AND W. A. CALDER. 1976. Body size and longevity in birds. *Condor* 78: 91–94.
- MCCLINTOCK, B. 1941. The stability of broken ends of chromosomes in *Zea mays*. *Genetics* 41:234–282.
- METZ, V. G., AND E. A. SCHREIBER. 2002. Great Frigatebird (*Fregata minor*). In *The Birds of North America*, no. 681 (A. Poole and F. Gill, Eds.). *Birds of North America*, Philadelphia.
- MEYNE, J., R. L. RATLIFE, AND R. K. MOYZIS. 1989. Conservation of the human telomere sequence (TTAGGG)_n among vertebrates. *Proceedings of the National Academy of Sciences USA* 86:7049–7053.
- NAKAGAWA, S., N. J. GEMMEL, AND T. BURKE. 2004. Measuring vertebrate telomeres: Applications and limitations. *Molecular Ecology* 13:2523–2533.
- OLOVNIKOV, A. M. 1973. A theory of marginotomy: The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *Journal of Theoretical Biology* 41: 181–190.
- PROWSE, K. R., AND C. W. GREIDER. 1995. Developmental and tissue-specific regulation of mouse telomerase and telomere length. *Proceedings of the National Academy of Sciences USA* 92:4818–4822.
- RUFER, N., T. H. BRÜMMENDORF, S. KOLVRAA, C. BISCHOFF, K. CHRISTENSEN, L. WADSWORTH, M. SCHULZER, AND P. M. LANSDORP. 1999. Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. *Journal of Experimental Medicine* 190:157–167.
- SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press, New York.
- SMOGORZEWSKA, A., J. KARLSEDER, H. HOLTGREVE-GREZ, A. JAUCH, AND T. DE LANGE. 2002. DNA ligase IV-dependent NHEJ of deprotected mammalian telomeres in G1 and G2. *Current Biology* 12:1635–1644.
- VAZIRI, H., W. DRAGOWSKA, R. C. ALLSOPP, T. E. THOMAS, C. B. HARLEY, AND P. M. LANSDORP. 1994. Evidence for a mitotic clock in human hematopoietic stem cells: Loss of telomeric DNA with age. *Proceedings of the National Academy of Sciences USA* 91:9857–9860.
- VLECK, C. M., M. F. HAUSSMANN, AND D. VLECK. 2003. The natural history of telomeres: Tools for aging animals and exploring the aging process. *Experimental Gerontology* 38: 791–795.
- VON ZGLINICKI, T., R. PILGER, AND N. SITTE. 2000. Accumulation of single-strand breaks is the major cause of telomere shortening in human fibroblasts. *Free Radical Biology and Medicine* 28:64–74.
- WATSON, J. D. 1972. Origin of concatameric T4 DNA. *National New Biology* 239:197–201.
- ZAKIAN, V. A. 1995. Telomeres: Beginning to understand the end. *Science* 270:1601–1607.
- ZAR, J. H. 1996. *Biostatistical Analysis*, 3rd ed. Prentice Hall, Upper Saddle River, New Jersey.
- ZEICHNER, S. L., P. PALUMBO, Y. FENG, X. XIAO, D. GEE, J. SLEASMAN, M. GOODENOW, R. BIGGAR, AND D. DIMITROV. 1999. Rapid telomere shortening in children. *Blood* 93:2824–2830.

Associate Editor: A. E. Burger