Telomeres and Longevity: Testing an Evolutionary Hypothesis

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Identifying mechanisms that underlie variation in adult survivorship provide insight into the evolution of life history strategies and phenotypic variation in longevity. There is accumulating evidence that shortening telomeres, the protective caps at the ends of chromosomes, play an important role in individual variation in longevity. Given that telomeres generally shorten with age, it was surprising to find that in a population of a long-lived seabird, Leach's storm petrel, telomeres appear to lengthen with age. This unique finding suggested that the longest lived individuals are able to elongate telomeres, an interpretation we call the "elongation hypothesis." Alternatively, the "selection hypothesis" states that the longest lived individuals start with the longest telomeres and variation in telomere length decreases with age due to the selective disappearance of individuals with short telomeres. In the same population in which evidence supporting both hypotheses was uncovered, we tested mutually exclusive predictions from the elongation and selection hypotheses by measuring telomere length with the telomere restriction fragment assay in hatchling and old, adult storm petrels. As previously found, adult birds had longer telomeres on average compared with hatchlings. We also found that 3 hatchlings had mean telomere lengths exceeding that of the most extreme old bird, old birds on average had longer initial telomere lengths than hatchlings, and the variance in mean telomere length was significantly greater for hatchlings than for old birds, all predicted by the selection hypothesis. Perhaps more surprisingly, the oldest adults also show little or no accumulation of short telomeres over time, a pattern unknown in other species. Long telomeres are thought to provide a buffer against cellular senescence and be generally indicative of genome stability and overall cell health. In storm petrels, because the progressive accumulation of short telomeres appears negligible, variation in telomere length at birth may be linked to individual variation in longevity.

Introduction

The concept of trade-offs is central to our understanding of the evolution of life histories. Differences within and among species in life history strategies are generally framed in terms of differences in the optimal investment in growth, reproduction, and self-maintenance. Variability in the prevention, generation, and repair of somatic damage between individuals plays out against a backdrop of environmental fluctuation resulting in phenotypic variation at young ages. Identifying mechanisms that underlie variation in adult survivorship should provide insight into the evolution of life history strategies and phenotypic variation in longevity. Telomere dynamics link molecular and cellular mechanisms with organismal processes such as growth and survival and, therefore, may be integral to such trade-offs (von Zglinicki 2002; Monaghan and Haussmann 2006).

Telomeres are found at the termini of eukaryotic chromosomes and function in the protection of DNA (Prowse and Greider 1995). Each time a cell divides, its telomeres shorten due to the end replication problem, and this is reflected by ever-shortening telomeres during organismal aging in most animals (reviewed in Sedivy 1998; Haussmann, Winkler et al. 2003). In both birds (Hall et al. 2004) and mammals (Jennings et al. 1999), faster growth rates result in greater telomere loss, and it has been suggested that changes in telomere lengths might provide the link between early growth conditions and the pace of deterioration later in life (Metcalfe and Monaghan 2003; Monaghan and Haussmann 2006). Telomeres are also particularly susceptible to free radicals that are thought to be major contributors to the aging process (Beckman and Ames 1998), and telomere shortening may provide a signal for knocking out cells with high levels of DNA damage (von Zglinicki 2002; di Fagagna et al. 2003). Once telomeres shorten to a critical length, a cell cycle checkpoint is triggered and cells enter a terminally nondividing state known as replicative senescence (Hornsby 2002). Telomeres can be elongated by the enzyme telomerase (Greider and Blackburn 1985), which is repressed in most normal adult somatic tissues because telomerase activation is necessary for cancer cells to maintain telomeres over multiple rounds of cell division (Forsyth et al. 2002). Thus, telomere shortening is thought to act as both an anticancer mechanism and contribute to the aging process (Campisi 2003; Hornsby 2006).

Although the contribution of telomere maintenance to organismal aging has been controversial, there is converging evidence from basic and clinical studies that telomere maintenance plays an important role in organismal longevity (Cawthon et al. 2003; Joeng et al. 2004; Haussmann et al. 2005). In the only prospective study of telomere length and longevity in humans, 60- to 97-year-old individuals with short telomeres had higher mortality than individuals with long telomeres (Cawthon et al. 2003). The survival difference was attributable in part to those with short telomeres having higher mortality from heart disease and infectious disease, lending support to the hypothesis that telomere shortening contributes to mortality in many age-related diseases. Joeng et al. (2004) demonstrated that *Caenorhabditis elegans* with experimentally lengthened telomeres through overexpression of HRP-1, a telomere-binding protein, lived longer and were more resistant to heat stress. Telomere length also appears to relate to survival in wild populations, as yearling female tree swallows (*Tachycineta bicolor*) with shorter than average telomere lengths were less likely to return to the breeding site in subsequent years than those with longer than average telomere lengths (Haussmann et al. 2005), suggesting that telomere maintenance is not only associated with late-life mortality (Haussmann et al. 2005). Recent human studies also show no clear association between telomere length and survival in humans greater than 85 years of age (Martin-Ruiz et al.

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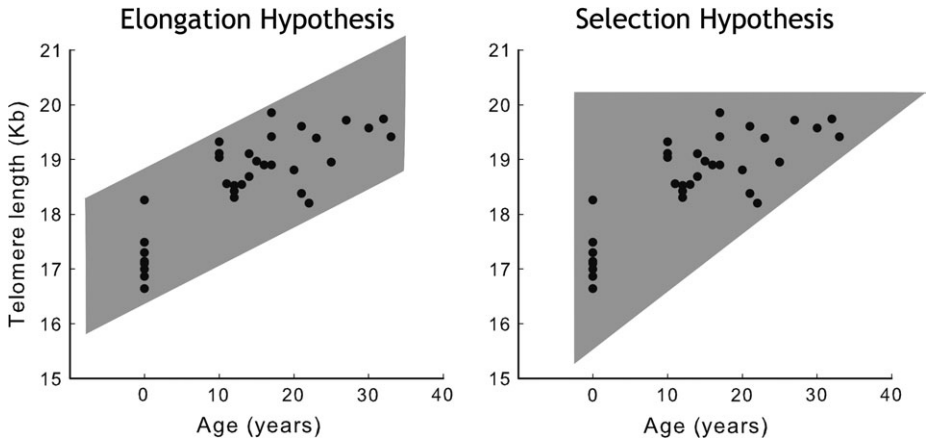

FIG. 1.—The relationship between telomere length and age predicted by elongation and selection hypotheses. Data points represent storm petrels measured in a previous cross-sectional study by Haussmann, Winkler et al. (2003). As predicted by both hypotheses, telomere length increases with age, even when hatchlings are removed from the analysis (Haussmann, Winkler et al. 2003). As predicted by the elongation hypothesis, variation in telomere length does not change with age. When the hatchlings are removed from the analysis, variation in telomere length decreases with age, as predicted by the selection hypothesis. The shaded areas represent data space where an individual of a certain age can possess a particular telomere length as predicted by the elongation and selection Hypotheses. Absolute mean telomere length values differ from those in the present study due to different electrophoretic methods.

2005; Bischoff et al. 2006). This suggests that telomere length may be a more informative biomarker for mortality in young- and middle-aged individuals rather than to the oldest individuals of a species (Baird 2006).

Recently, the rate of telomere shortening was shown to be negatively correlated with organismal life span (Haussmann, Winkler et al. 2003), suggesting that longer lived species better maintain their telomeres compared with shorter lived ones. In one species, Leach's storm petrel (*Oceanodroma leucorhoa*), a long-lived seabird that lives 4 times longer than expected based on body mass (Haussmann et al. 2007), telomere length did not shorten with age but instead appeared to lengthen across the bird's life span (Haussmann, Winkler et al. 2003). This finding, novel among all animals tested, provides further evidence that telomere maintenance is related to cellular and organismal longevity. Specifically, it suggests that the longest lived storm petrels are those able to lengthen telomeres with age, perhaps, due to the high levels of telomerase activity found in these birds (Haussmann et al. 2007). The apparent lengthening of telomeres with age in this species we term the "elongation hypothesis." Confirmation of this hypothesis would be the first demonstration of telomere elongation in any species (Choi 2003) and may offer insight into the evolution of the exceptionally long mass-adjusted life span in this bird.

An alternative explanation for the apparent lengthening of telomeres with age may be due to intrinsic phenotypic differences among individuals, a process recently demonstrated with respect to age-specific breeding success in this same population of storm petrels. Age-specific breeding success has been demonstrated in many species, with performance lower in young than in older breeders (Pyle et al. 2001; Cichon 2003). Three hypotheses have been raised to explain this general phenomenon, the "experience hypothesis" based on age-specific reproductive experience (Curio 1983), the "effort hypothesis" based on age-specific reproductive effort (Forslund and Part 1995), and the "selection hypothesis" based on progressive asymmetric disappearance of poor-quality phenotypes (Curio 1983; Forslund and Part 1995). These 3 hypotheses make distinct predictions with regard to the relationship between longevity and reproductive success in the initial breeding attempts. Specifically, the effort hypothesis predicts a negative correlation, the experience hypothesis predicts no correlation, and the selection hypothesis predicts a positive correlation between early reproductive success and life span (Mauck et al. 2004). These predictions were tested by examining breeding histories for more than 6,000 breeding storm petrels. Reproductive success in the first 2 breeding attempts was positively correlated with longevity, supporting the selection hypothesis and suggesting intrinsic phenotypic differences among individuals (Mauck et al. 2004).

These results offer an alternative explanation for the apparent lengthening of telomeres with age under the general framework of the selection hypothesis (Forslund and Part 1995; Part 1995). The selection hypothesis depends on the presence of initial variation in the trait of interest, followed by selection on advantageous phenotypes (Forslund and Part 1995). In this case, initial telomere length, as measured in hatchling storm petrels, should be highly variable. Initial telomere length is the result of zygote telomere length and maintenance mechanisms (Graakjaer et al. 2004) acting on that telomere length until hatching. If initial telomere length of hatchlings is correlated with longevity and only hatchlings with long initial telomere lengths survive to old age, then mean telomere length would increase as individuals with short telomeres are culled from the population. Thus, the selection hypothesis can also explain the observed positive correlation of telomeres with age in storm petrels. Confirmation of this hypothesis suggests that there is selective mortality based in part on telomere length, and those individuals starting with longer telomeres may be a particularly resilient subset that are more likely to reach old age.

We tested mutually exclusive predictions derived from the elongation and selection hypotheses with regard to

telomere length in hatchling and adult storm petrels (fig. 1) in the same population in which evidence supporting both hypotheses was uncovered. If telomeres lengthen with age as predicted by the elongation hypothesis, then in even a very large sample of hatchlings (see Methods), not a single hatchling should have telomeres longer than or equal to the longest telomeres measured in the oldest storm petrels. Alternatively, the selection hypothesis predicts that in a very large sample of hatchlings, we will find at least one hatchling with telomere lengths greater than or equal to the longest telomeres measured in the oldest storm petrels. The elongation hypothesis also predicts that all hatchlings begin with relatively short telomere lengths and variation will remain relatively constant with age as individuals lengthen telomeres or, perhaps, increase with age depending on the dynamics of telomere maintenance (fig. 1) (von Zglinicki 2000; Monaghan and Haussmann 2006). In contrast, a key prediction from the selection hypothesis is that variance will decrease with age in any measured variable that influences quality as low-quality individuals are culled from the population. Thus, variation in telomere length should decrease with age. Specifically, variance in telomere length among hatchlings should be greater than variance in telomere length among old individuals (fig. 1).

If initial telomere length alone is, in fact, linked to organismal longevity, then those individuals born with longer telomeres may be at an advantage in the population. However, additional variation in telomere length over an individual's life span can be attributed to telomere dynamics. Telomere length regulation balances shortening events, including cell division and oxidative damage (von Zglinicki et al. 2000; Epel et al. 2004) with maintenance events that protect telomeres, such as telomerase and antioxidants (Bodnar et al. 1998; Ostler et al. 2000; Saretzki et al. 2003). Telomere shortening is inevitable in most species and eventually can lead to the accumulation of critically short telomeres (Hemann et al. 2001). Critically, short telomeres are indicative of telomere dysfunction that leads to cellular senescence (Herbig et al. 2006) and the loss of organismal viability (Blasco 2005; Canela et al. 2007), so individuals with a higher proportion of short telomeres may be at a higher mortality risk. Thus, both initial telomere length and the proportion of short telomeres are likely to be important indicators of the probability that a cell will become senescent.

In a sample of cells, telomere length is variable because cells are descendants from stem cells of different replicative history (Juola et al. 2006; Drummond et al. 2007). The telomere restriction fragment (TRF) assay has proven to be one of the most powerful and informative telomere length analysis technologies because it allows for the measurement of a population of telomeres for each individual (Baird 2006). Measurement of the entire population of telomeres within a sample of cells is termed genome-wide telomere length (Baird et al. 2006) or global telomere length (Rashid-Kolvear et al. 2007) and offers information on telomere variability within an individual- and species-specific regulation of telomere length (Baird et al. 2006).

Another advantage of the TRF assay is that it allows for the measurement of subsets of the telomere population distribution (Haussmann and Mauck 2007). In hatchlings, the subset of the longest telomeres in the telomere population distribution most closely approximates initial telomere length before cell division and oxidative damage lead to telomere erosion and in some cases critically short telomeres. The proportion of critically short telomeres in the telomere population distribution, rather than mean telomere length, is the best measure of those telomeres that may signal cellular senescence (Herbig et al. 2006; Canela et al. 2007). In this study, we compared genome-wide, initial, and critically short telomere length of old and hatchling storm petrels using the TRF assay to test predictions from the elongation and selection hypotheses.

Methods

Birds

Leach's storm petrels were from a breeding colony of about 20,000 pairs of Leach's storm petrels at the Bowdoin College Scientific Station on Kent Island, New Brunswick, Canada (66°45′ W, 44°35′ N). This population has been monitored annually since 1953 and birds of all ages, up to 35 years, currently breed at the colony (Huntington et al. 1996). Leach's storm petrels of both sexes usually delay breeding until 4 or 5 years of age, then breed yearly for up to 30 years. Estimates of adult survival range from 0.86 to 0.93 (Huntington et al. 1996) and may be closer to 0.89 (Mauck RA, unpublished data). The female lays a single egg each year and the male and female share incubation duties during a 40- to 44-day incubation period. The nestling is brooded for about 5 days, after which it remains alone in the burrow for another 55–65 days before fledging (Huntington et al. 1996). This study population is the same system in which both telomere lengthening and age-specific breeding success were examined (Haussmann, Winkler et al. 2003; Mauck et al. 2004).

Samples

We located 24 breeding adult Leach's storm petrels of at least 25 years of age with a maximum of 33 years of age and monitored hatch date for 160 hatchlings during June and July 2006. We collected 70 μl whole blood from each adult during incubation and from each hatchling within 2 days posthatch. Whole blood was centrifuged at 3,500 g for 10 min, and plasma was removed. The remaining blood cells were frozen in 90% fetal bovine serum and 10% dimethyl sulfoxide buffer and stored at −20 °C until DNA extraction in the laboratory.

TRF Assay

The TRF assay and TRF analysis followed Haussmann and Mauck (2007). Briefly, DNA was extracted in 0.8% agarose plugs, and plugs were treated with proteinase K overnight at 50 °C. Following protein digestion, plugs were restriction digested with 15 U of *Hin*fI, 75 U of *Hae*III, and 40 U of *Rsa*I at 37 °C. One-half of each plug was loaded into a 0.8% nondenaturing agarose gel. DNA was separated using pulsed field gel electrophoresis (3 V/cm, 0.5–7.0 s

switch times, 14 °C) for 21 h, followed by in-gel hybridization at 37 °C overnight with a radioactive-labeled telomere-specific oligo $(CCCTAA)_4$. Hybridized gels were placed on a phosphorscreen (Amersham Biosciences, Buckinghamshire, UK), for 3 days.

TRF Analysis

The phosphorscreen was scanned on a Storm 540 Variable Mode Imager (Amersham Biosciences) to visualize the telomeres. We used densitometry (ImageQuant 5.03v and ImageJ 1.37v) to determine the position and strength of the radioactive signal in each of the lanes. Average labeled telomere length in each lane is calculated using the formula: $L = \sum(\mathrm{OD}_i \times L_i) / \sum(\mathrm{OD}_i)$, where OD_i is the densitometry output at position i and L_i is the length of the DNA (bp) at position i.

Circulating erythrocytes are descendants from hematopoietic stem cells of different replicative history, so telomere lengths measured in a sample of erythrocytes are variable. Thus, the telomere distribution in a TRF assay represents a population of telomeres for each individual. We measured different subsets of the telomere distribution (fig. 2). We first measured the entire telomere distribution to determine genome-wide telomere length by calculating mean telomere length over the entire gel lane (fig. 2*a*). To approximate initial telomere length, we measured the longest detectable telomeres for each individual. We started at a higher molecular weight than the telomere distribution for each individual, and then moving down the distribution, we determined the highest molecular weight where optical density values were above background (fig. 2*b*). Adult birds have longer replicative histories than hatchlings, so our approximation may underestimate actual initial telomere length of old birds. However, some cells of the hematopoietic stem cell population divide very rarely (in humans once every 1–2 years, Drummond et al. 2007). Our measure of initial telomere length depends on detecting any telomere signal rather than an average telomere length. To the extent that maximum telomere length indicates initial telomere length, this measure should provide a useful estimate of initial telomere length. To investigate the critically short telomeres, we calculated the proportion of the entire distribution of telomeres for each individual that consisted of telomeres with length less than 6 kb. We defined telomeres <6 kb as critically short telomeres because telomeres shorter than this length in humans, another long-lived species, lead to telomere dysfunction (Canela et al. 2007). Specifically, we calculated the area under the curve of the x axis (a linear measure of the gel in pixels) and the y axis (the optical density). Then, for each individual, we calculated the area under the optical density curve below 6 kb divided by the area under the curve for the entire distribution (fig. 2*c*).

We measured telomere length in nucleated erythrocytes, which likely reflect telomere lengths of the hematopoietic stem cells (Vaziri et al. 1994), because blood cells do not divide while in circulation. We used peripheral blood cells because they are easily obtained from animals in the field in a relatively noninvasive manner and only a few drops of blood are necessary for TRF analysis (Haussmann and Vleck 2002; Nakagawa et al. 2004). The majority of our understanding about telomere dynamics as a function of age was gained from studies on peripheral blood cells due to the above reasons (Baird 2006). Although telomere lengths vary between tissues (Forsyth et al. 2002), individuals with relatively short or long telomeres in one somatic tissue type express relatively short or long telomeres in other somatic tissue types (Martens et al. 1998; Okuda et al. 2002).

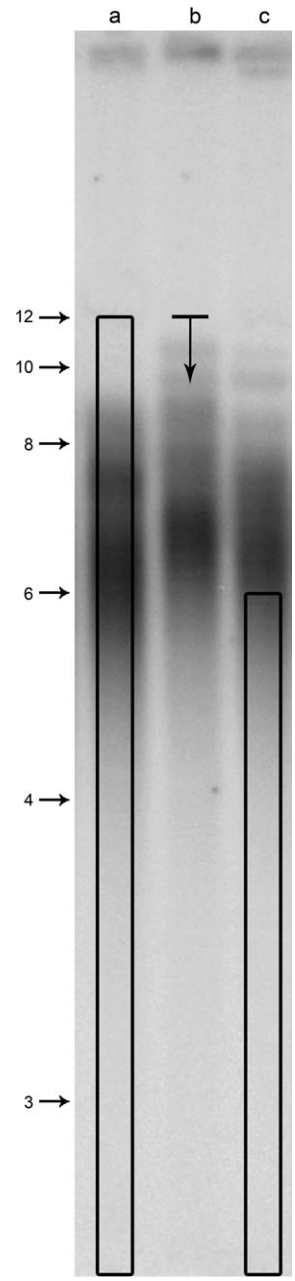

FIG. 2.—Telomere gel with analysis windows. Entire distribution of telomeres representing genome-wide telomere length (*a*) includes telomere lengths that range from approximately 13–1.2 kb. Window representing the longest telomeres (*b*) includes maximum detectable telomere lengths above background optical density. Shortest telomeres (*c*) represent telomere lengths below approximately 6 kb. Lanes *a* and *c* are hatchlings, whereas *b* is an old bird. Location of 1 kb plus size markers (in kb) are shown along the left-hand side of the gel.

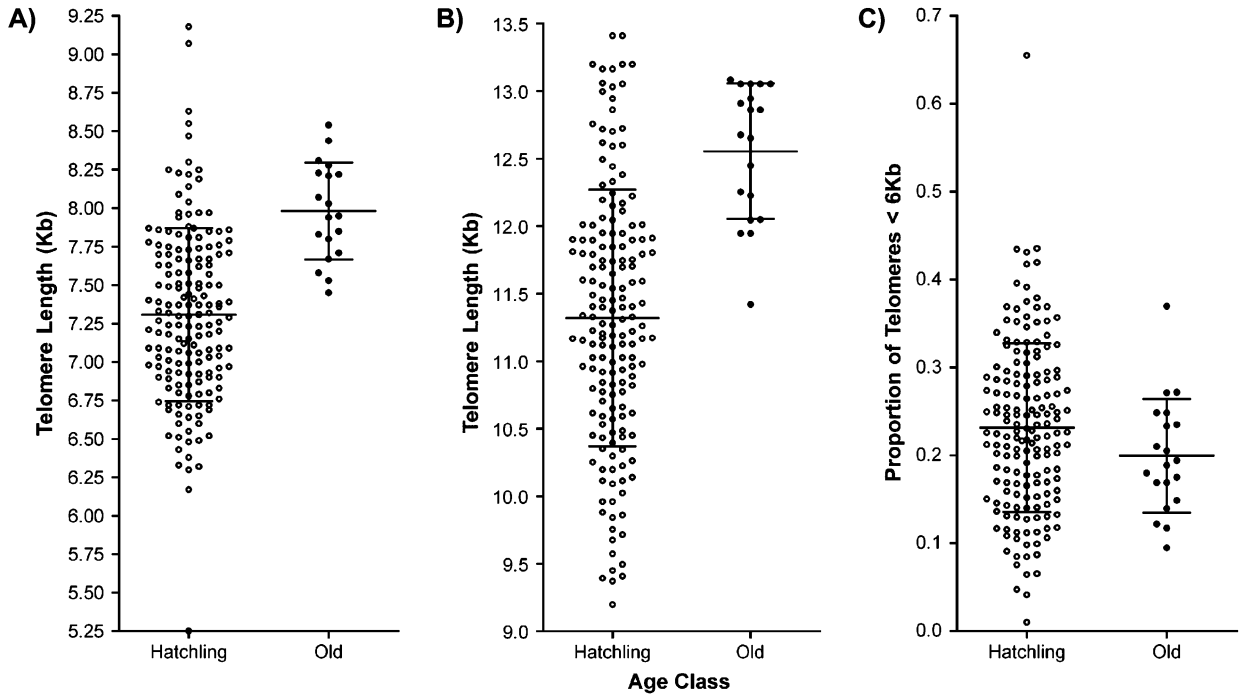

FIG. 3.—Distribution of telomere lengths by age class. Each point represents a measure of telomere length for one individual hatchling (open circles) or old (closed circles) storm petrel. Measures include (*A*) genome-wide mean telomere length (hatchling: 7.31 ± 0.56 standard deviation [STD] kb; old: 8.00 ± 0.32 STD kb), (*B*) longest telomere length (hatchling: 11.32 ± 0.95 STD kb; old: 12.55 ± 0.50 STD kb), and (*C*) proportion of the distribution of all telomeres that includes only the shortest (hatchling: 0.23 ± 0.096; old: 0.20 ± 0.065) telomeres (fig. 2) for 19 individuals at least 25 years old (old) and 167 individuals less than 3 days old (hatchling). Mean telomere length is calculated using the formula: $L = \sum(\mathrm{OD}_i \times L_i)/\sum(\mathrm{OD}_i)$, where OD_i is the densitometry output at position i and L_i is the length of the DNA (bp) at position i. Horizontal lines represent mean and 1 standard deviation for each distribution.

Sample Size Calculation

Only 1% of storm petrels chicks return to breed on Kent Island (Huntington et al. 1996). Combined with delayed breeding, this lack of philopatry makes longitudinal studies particularly difficult. An initial sample of 100 chicks would yield a single breeding adult 5 years later. Aside from the practical issues imposed by delayed breeding, an initial sample of 10,000 chicks would be needed to obtain 100 usable adults 5 years later, of which 10% would be lost each year. However, a sample size of 100–200 chicks can provide sufficient information about telomere length in young birds to test predictions from the selection hypothesis.

Support for the selection hypothesis requires identifying at least one hatchling with mean telomere lengths exceeding the oldest bird (33 years) for which telomeres were measured in the previous study (Haussmann, Winkler et al. 2003). The elongation hypothesis requires that we find 0 hatchlings with telomeres longer than those previously found in the oldest individuals. We calculated the sample size needed to support the elongation hypothesis (i.e., finding 0 cases of long telomeres in hatchlings) with probabilities of falsely rejecting the null (β error) at the 0.05 level. To do this, we calculated a cumulative binomial distribution with the following parameters: number of successes = 0, probability of success = 0.03 (the probability of living to 33 years assuming 0.89 annual survival probability). For β = 0.05, we must sample 160 hatchlings. Hatchlings were not offspring from the old 24 breeding adults we sampled, and all hatchlings were sampled from different parents to ensure independent data points (Hurlbert 1984).

Statistical Analysis

Telomere measurements within groups were normally distributed, and we used parametric statistics to make between group comparisons. To test for differences in mean values, we used Welch's *t*-test to correct for unequal variances between old adult and hatchling telomere measurements. We used an *F*-test to test the prediction of unequal variance between old and hatchling telomere measurements. All tests were 2 tailed.

Results and Discussion

Genome-wide mean telomere length (fig. 2*a*) of old birds was significantly greater than in hatchlings (Welch's corrected *t*-test of unequal variance, $t = 7.962$, degrees of freedom [df] = 32, $P < 0.0001$; fig. 3*A*). Three hatchlings had mean telomere lengths exceeding that of the most extreme old bird, and the variance in genome-wide mean telomere length was significantly greater for hatchlings than for old birds (2-tailed *F*-test for unequal variance, $F = 3.17$, $P = 0.0065$).

If old storm petrels start with longer telomeres, then initial telomere length may be linked with reaching old ages.

Under this assumption, we determined the length of the longest detectable telomeres for each individual (fig. 2*b*). We found that, on average, initial telomere length was greater in old birds compared with hatchlings (Welch's corrected *t*-test of unequal variance, $t = 9.045$ df $= 35$, $P < 0.0001$; fig. 3*B*), variance was less in old birds compared with hatchlings (2-tailed *F*-test for unequal variance, $F = 3.60$, $P < 0.003$), and 7 hatchlings exceeded maximum telomere length of any old bird.

Initial telomere length is established in the zygote of individuals (Graakjaer et al. 2004), and there is a strong genetic component (heritability, ~80%) to telomere length in humans (Slagboom et al. 1994; Jeanclos et al. 2000; Vasa-Nicotera et al. 2005). For tree swallows, estimated heritability of telomere length prior to fledging is 87%, based on full sibling relationships (Haussmann MF, unpublished data). This suggests that initial lengths of an individual's telomeres are in part dependent on the telomere lengths of their parents' germ cells. If telomere length in storm petrel is also highly heritable then, under the selection hypothesis, offspring with parents who have long initial telomere length are at an advantage within the population relative to those with short initial telomere length.

Together, these results provide strong support for the selection hypothesis that predicts wide initial variation in the trait of interest, followed by selection on advantageous phenotypes, resulting in reduced variation in the trait (Forslund and Part 1995). Over both genome-wide telomere length and initial telomere length, we see that the mean value for old individuals is greater than that for young birds and old birds also align with the subset of young birds with the longest telomeres. A key result is that in both cases a small proportion of young birds exceeded even the oldest individuals measured, suggesting that the relatively long telomeres possessed by the oldest storm petrels may represent a subset of the initial storm petrel population distribution of telomere lengths. If so, then one component of attaining old age in this species may be the possession of long telomeres (Cawthon et al. 2003; Joeng et al. 2004; Haussmann et al. 2005).

Interestingly, a greater proportion of hatchlings than expected had initial telomere lengths in the range of old storm petrels. Given the range of adult survivorship estimates (0.86–0.93; Huntington et al. 1996), 6–26 of 160 hatchlings would be expected to live at least 25 years. If initial telomere length alone accounts for variation in longevity, then at most 26 hatchlings should have telomere lengths in the range of the old birds. We found 32 with their longest telomere lengths in that range. As previously mentioned, the longer replicative histories of adult hematopoietic stem cells suggest that adult initial telomere length is likely to be slightly longer than what we measured here. If so, this would shift the adult initial telomere length up and eliminate some of the hatchlings within the adult range making the number of hatchlings with telomere lengths in the range of old birds closer to what we predicted.

Longevity is positively correlated with lifetime reproductive success in this species (Mauck et al. 2004). Therefore, if initial telomere length alone accounts for variation in longevity, then selection on telomere length should result in only long initial telomere length, with short telomeres being eliminated completely. This is not the case, and there have been no reports of trade-offs between telomere length and fecundity. Although a number of human studies have reported a strong genetic component to telomere length (Slagboom et al. 1994; Jeanclos et al. 2000; Vasa-Nicotera et al. 2005), telomere length is highly variable at birth (Okuda et al. 2002) and during adult life (Valdes et al. 2005). One recent heritability study suggests the importance of environmental influences (Andrew et al. 2006) on telomere length. The analysis of both monozygotic and dizygotic human twin pairs provided an estimate of h^2 of 36%, but once shared environmental effects were accounted for h^2 rose to 90% (Andrew et al. 2006). Therefore, environmental effects appear to play a key role in shaping telomere length. Environmental effects related to growth seem to be particularly important and can occur before birth or hatching. Compared with mothers fed a control diet, pregnant rats (*Rattus norvegicus*) fed a low protein diet had offspring with growth retardation during fetal life followed by postnatal catch-up growth associated with shorter telomeres and shorter life span. Taken together, these results suggest that although initial telomere length is important, some variation in telomere length is due to environmental sources, perhaps, before the egg hatches.

The rate of telomere shortening also varies markedly among individuals in association with the level of oxidative stress that they have experienced (von Zglinicki 2003; Epel et al. 2004) and, perhaps, the demonstrated capacity of telomerase and antioxidants to maintain and protect telomeres (Bodnar et al. 1998; Ostler et al. 2000; Saretzki et al. 2003). Telomere length and shortening rates are correlated to levels of oxidative stress, and an individual's telomere length at a certain age is largely dependent on the amount of oxidative stress accumulated over its lifetime (von Zglinicki 2000). Telomere shortening eventually leads to the increase of critically short telomeres, and it is this subset of telomeres, rather than genome-wide telomere length, that is indicative of telomere dysfunction that can lead to cellular senescence (Herbig et al. 2004) and the loss of organismal viability (Blasco 2005). Recent evidence shows that the number of telomere-dependent senescent cells in old individuals is much higher than previously thought (Baird 2006). Herbig et al. (2006) observed an exponential increase in the number of telomere-dependent senescent cells with age in baboons, which reached a value of 30–35% in the oldest individuals. This study provided evidence that not only do telomere-dependent senescent cells exist in vivo but also they increase with age and can compromise up to one-third of the total cell population (Herbig et al. 2006).

So, although the oldest birds in the storm petrel population may have started with long telomeres, we next asked whether they were better able to avoid the accumulation of critically short telomeres that indicate high levels of oxidative stress. If so, this suggests that these old birds were able to maintain telomere integrity throughout their lifetimes. To test this post hoc hypothesis, we examined the area of the distribution where critically short telomeres are found (<6 kb; Canela et al. 2007, fig. 2*c*). For many species, including birds (Haussmann, Vleck, et al. 2003; Haussmann, Winkler et al. 2003; Haussmann and Mauck

2007) and humans (Canela et al. 2007), older individuals have a larger proportion of short telomeres relative to young individuals. If, however, the longest lived storm petrels not only start with long telomeres but also have better than average telomere maintenance mechanisms, then the oldest birds should have an equal proportion of telomeres classified as critically short compared with the hatchlings. In fact, we found that the proportion of telomeres in that region (fig. 3*C*) was less for old birds than for hatchlings (Welch's corrected *t*-test, $t = 1.97$, df = 30, $P = 0.0586$), particularly when the extreme outliers (fig. 3*C*) from each age class is removed (Welch's corrected *t*-test, $t = 7.64$, df = 32.02, $P = 0.009$). When we limited the comparison to those hatchlings that begin with long telomeres, we find no difference between old birds and these old-like hatchlings with regard to critically short telomeres (Welch's corrected *t*-test, $t = 1.71$, df = 49, $P = 0.86$).

Given that the old individuals in this study have dealt with the effects of oxidative stress and DNA replication for over 25 years versus just 2 days for hatchlings, this is a remarkable finding. It suggests that telomere shortening in this long-lived species occurs very slowly, or possibly not at all, a finding consistent with the negative relationship between life span and rate of telomere shortening across species (Haussmann, Winkler et al. 2003). The longest lived individuals may be associated not only with long initial telomere lengths but also with superior telomere maintenance mechanisms as well. In fact, the primary mode of telomere restoration is through the enzyme telomerase (Greider and Blackburn 1985), and Leach's storm petrels express bone marrow telomerase at high levels throughout life, which may account for the slower rates of erythrocyte telomere shortening in this longer lived bird (Haussmann et al. 2007). Another intriguing possibility is that free radical generation is at low enough levels or that antioxidant defense is at high enough levels that this species accumulates very little oxidative damage over its life span. A longitudinal study of individuals would allow for an exploration of how other factors, such as DNA damage susceptibility, DNA repair capabilities, and shelterin proteins that controls the synthesis of telomeric DNA by telomerase (de Lange 2006), affect telomere dynamics in this species. Whether natural selection has acted on telomerase or these other mechanisms to adjust the telomere dynamics and thereby modify life span in this extremely long-lived bird species awaits further study.

The effects of both initial telomere length and shortening caused by telomere dynamics combine to determine telomere length at any given age in an individual. The selection hypothesis with regard to telomeres is readily evident in Leach's storm petrels because the apparent superior telomere maintenance mechanisms render telomere shortening insignificant. This allows easy detection of the progressive disappearance of short initial telomere phenotypes from the population (fig. 1). However, previous work has shown that telomere length is also related to mortality risk in relatively short-lived species like worms (Joeng et al. 2004) and tree swallows (Haussmann et al. 2005). This suggests that in regard to telomeres, the selection hypothesis may hold in other species, albeit through a more complex process. The rate of telomere shortening is negatively correlated with organismal life span in birds and mammals, so that short-lived species lose more telomeres each year than long-lived species (Haussmann, Winkler et al. 2003). In species showing telomere shortening with age, both initial telomere length and telomere shortening may be related to survival, and the loss of telomeres over time (Haussmann, Winkler et al. 2003) can mask the disappearance of short telomere phenotypes.

Conclusion

Our results provide strong evidence for the selection hypothesis with regard to telomeres and longevity in Leach's storm petrel. Older birds not only had longer genome-wide mean telomere length, compared with hatchlings, but they also appear to have on average longer initial telomere length, as reflected in the maximum detectable telomere lengths in each individual's entire distribution. Long telomeres may provide a buffer against cellular senescence (di Fagagna et al. 2003; Herbig et al. 2006) or be generally indicative of genome stability or overall cell health (von Zglinicki 2002; Cawthon et al. 2003; Epel et al. 2004). Perhaps, more surprising is the novel finding that the oldest individuals in this population show little or no accumulation of short telomeres over time, a pattern unknown in other species. Managing the pace of telomere loss and restoration may be important to life history trade-offs as age-independent telomere length predicts longevity and lifetime reproductive success in birds. In species with apparent telomere shortening, both initial telomere length and telomere dynamics are likely to determine the onset of telomere dysfunction. The relative importance of each factor may depend on where a species falls in the life history continuum. In short-lived species, telomere dynamics and shortening events may account for most of the variation in telomere erosion, whereas initial telomere length may play a more central role in long-lived species. In storm petrels, long telomeres combined with superior cellular maintenance mechanisms may provide a molecular explanation for phenotypic variation in longevity in this long-lived species.

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