THE ROYAL SOCIETY

Received 19 January 2004
Accepted 9 April 2004
Published online 15 June 2004

Telomere loss in relation to age and early environment in long-lived birds

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Shortening of telomeres, specific nucleotide repeats that cap eukaryotic chromosomes, is thought to play an important role in cellular and organismal senescence. We examined telomere dynamics in two long-lived seabirds, the European shag and the wandering albatross. Telomere length in blood cells declines between the chick stage and adulthood in both species. However, among adults, telomere length is not related to age. This is consistent with reports of most telomere loss occurring early in life in other vertebrates. Thus, caution must be used in estimating annual rates of telomere loss, as these are probably not constant with age. We also measured changes within individuals in the wild, using repeat samples taken from individual shags as chicks and adults. We found high inter-individual variation in the magnitude of telomere loss, much of which was explained by circumstances during growth. Individuals laying down high tissue mass for their size showed greater telomere shortening. Independently of this, individuals born late in the season showed more telomere loss. Early conditions, possibly through their effects on oxidative stress, appear to play an important role in telomere attrition and thus potentially in the longevity of individuals.

Keywords: ageing; early growth conditions; lifespan; longitudinal studies; senescence

1. INTRODUCTION

Telomeres are repetitive DNA sequences that cap the ends of eukaryotic chromosomes. In the absence of the enzyme telomerase, telomeres shorten at cell division, eventually reaching a critical length that triggers cellular senescence (Vaziri *et al.* 1994; Allsopp & Harley 1995). Telomere shortening has been linked to tissue and organismal ageing (Campisi 1996; Kirkwood 2002; Bird *et al.* 2003). Initial telomere length appears to be partly determined by genetic influences (Slagboom *et al.* 1994), but other factors, particularly levels of oxidative stress, also influence the rate of telomere loss (Jennings *et al.* 2000; Von Zglinicki 2002). Thus, variation in the rate of telomere loss may relate to factors that influence oxidative damage, such as nutrition or growth (Jennings *et al.* 1999, 2000; Metcalfe & Monaghan 2001, 2003).

Age-related telomere attrition has been demonstrated in a variety of mammalian tissues (e.g. Harley *et al.* 1990; Coviello-McLaughlin & Prowse 1997). Recent studies in birds have examined telomere length as a potential biomarker of individual age, and in relation to interspecific variation in lifespan (Haussmann & Vleck 2002; Haussmann *et al.* 2003*a*,*b*). Old birds display shorter blood cell telomeres in the domestic chicken (*Gallus domesticus*) (Venkatesan & Price 1998; Delany *et al.* 2000), zebra finch (*Taeniopygia guttata)*, tree swallow (*Tachycineta bicolor*), Adélie penguin (*Pygoscelis adeliae*) and common tern (*Sterna hirundo*) (Haussmann & Vleck 2002; Haussmann *et al.* 2003*b*). As in most investigations of changes in telomere length with age, these avian studies are based on comparisons of telomere length among individuals of differing ages (cross-sectional analysis), because repeat measures of known-age individuals across a sufficient time span are very difficult to obtain, especially from long-lived organisms in the wild.

Cross-sectional data may not accurately reflect changes within individuals over time (Arking 1998). The phenotypic composition of age classes may differ as a result of selective mortality. In addition, environmental changes can be confounded with the effects of age; whole cohorts of offspring can be influenced by common natal environmental conditions (Lindström 1999; Reid *et al.* 2003). Furthermore, differences in individual rates of change cannot be examined in cross-sectional studies, making it difficult to identify factors influencing the rate of loss. There have been only two longitudinal studies so far of age-related telomere change in healthy individuals, both in mammals (humans (Zeichner *et al.* 1999) and domestic cats (Brummendorf *et al.* 2002)). These studies show that measurable telomere shortening takes place within individuals and, in line with human cross-sectional work (Frenck *et al.* 1998; Rufer *et al.* 1999; Friedrich *et al.* 2001), that telomere loss occurs at a higher rate early in life. Thus, conditions during juvenile life may be particularly important in determining telomere lengths in adulthood.

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In this study, we measured blood cell telomere length in samples from free-living, known-age individuals in two long-lived seabirds, the European shag (*Phalacrocorax aristotelis*) and the wandering albatross (*Diomedea exulans*). We examined telomere length in relation to age and sex where this was known. In shags, we investigated potential cohort influences on telomere length. We also examined telomere change within individuals in shags sampled as chicks and again as adults, and the extent to which conditions experienced during growth relate to subsequent telomere loss.

2. METHODS

(a) *Blood samples*

Blood was collected from shags on the Isle of May, Scotland (56°11′ N, 02°33′ W) under UK Home Office licence, during the 1997, 1998, 1999, 2001, 2002 and 2003 breeding seasons. Blood samples from wandering albatrosses were collected on Bird Island, South Georgia (54° S, 38° W), during the 2000 breeding season. Blood was taken by superficial venipuncture of the tarsus. Chick samples were stored as whole blood, whereas adult samples were separated into plasma and cells by centrifugation. Adult albatross samples were separated further into red and white blood cells. All samples were stored between −20 °C and −80 °C until DNA could be extracted.

Shag chicks were sexed from blood using a PCR-based method (Griffiths *et al.* 1998). Adults of both species were sexed by field observation. The laying dates of shags were determined by regular nest checks and by counting back through the laying sequence (eggs are laid every third day). At the time of blood sampling, birds were also weighed, and structural size measures (head and bill length, wing length, tarsus length) were taken.

Twelve pairs of apparent siblings (i.e. from the same brood) occurred among shag chicks for which telomere length was measured. However, the difference in telomere length between siblings did not differ from that between randomly assigned non-sibling pairs ($t_{22} = 1.72$, $p > 0.05$). The values of individuals, rather than brood averages, have been used in analyses. Out of the adult shags for which we could check the presence of siblings (86%), none had a sibling in the data. The remaining 14% were either ringed as adults or siblings were not recorded at ringing. Wandering albatrosses lay one egg at each breeding attempt, and because these samples were collected in a single year, no siblings were measured in the chick samples. Sixteen shag chicks, from different nest sites and therefore presumably unrelated, were measured for the second time as adults in 2003 at 2, 5 or 6 years old.

(b) *Measurement of telomere restriction fragments*

DNA was isolated from whole blood for chicks of both species and for adult shags, and from the red blood cell portion of adult albatross samples. Avian red blood cells are nucleated and because this forms the largest portion of the blood, all samples will represent red cell telomere lengths. Samples were digested with proteinase K before DNA extraction by standard procedures. Telomere restriction fragment (TRF) length was measured by Southern blot hybridization following the protocol outlined in the TeloTAGGG Telomere Length Assay Kit. TRF smears were transferred from Southern blots to autoradiography film. After scanning each image, the intensity of TRF smears at different molecular sizes was measured using TOTALLAB software. Mean TRF length per lane was calculated using the formula: $\text{meanTRF}L = \Sigma(\text{OD}_1)/\Sigma(\text{OD}_1 L_1)$, where OD_1 and L_1 are signal intensity and TRF length, respectively, at position 1 on the image. The background signal was subtracted from the signal intensity of each lane before calculation. Analysis was carried out blind with respect to donor age and sex, and average values from two lanes per sample were used in analyses. Mean TRF lengths measured independently by a second observer corresponded well (shags, $r = 0.97$, $n = 165$, $p < 0.001$).

(c) *Natal environmental conditions*

Albatross samples were collected in a single year, so all age-matched individuals have the same birth year. However, shag samples were taken in six different years, enabling us to examine potential cohort or environmental effects. We compiled measures of environmental conditions in each hatching year for shags from long-term colony productivity data collected on the Isle of May, using the first factor from a principal components analysis (PCA) as an indicator of conditions that year. We investigated whether telomere length was related to conditions or timing in the year of birth, and whether including hatching year as a variable affected the relationship between age and telomere length. Electronic Appendix A gives further details of the methods.

3. RESULTS

(a) *Cross-sectional analyses of telomere length*

Telomere length in shags ranged from 6.1 to 14.1 kb and in albatrosses from 6.6 to 14.2 kb. In cross-sectional data taking adults and chicks together, telomere length in both species appeared to decline with age. Shag chick telomeres were 10.7 ± 0.1 kb long (mean ± 1 s.e.m.), significantly greater than the adult mean of 8.4 ± 0.2 kb, and there was no significant effect of, or interaction with, sex (general linear model (GLM), age category chick or adult: $F_{1,149} = 123.24$, $p < 0.001$, mean difference (95% confidence interval (CI)) = 2.3 (1.9, 2.7) kb; sex: $F_{1,148} = 0.003$, $p = 0.96$; interaction term: $F_{1,147} = 0.65$, $p = 0.42$). Average telomere length of albatross chicks was 12.6 ± 0.2 kb and in this species mean adult telomeres were also significantly shorter at 9.9 ± 0.2 kb ($F_{1,91} = 86.64$, $p < 0.001$, mean difference (95% CI) = 2.7 (2.1, 3.3) kb). Again, there was no effect of sex on telomere length in adult albatrosses ($F_{1,60} = 1.97$, $p = 0.17$), and neither the interaction nor age were significant when sex was included in a GLM ($p > 0.05$). Telomere length did not correlate with age among shag ($r_S = -0.17$, $n = 90$, $p = 0.12$) or albatross ($r_S = -0.22$, $n = 31$, $p = 0.24$) chicks. When only adults were considered, there was no significant effect of age on telomere length in either shags or albatrosses (figure 1). Thus, in these species, the apparent decline in telomere length with age is largely a result of a difference between adults and chicks, rather than any consistent pattern of change in telomere length across the age classes.

In shags, year of hatching had no significant effect on telomere length in adults or chicks, nor did it interact with age category (GLM age category adult or chick when hatching year term included: $F_{1,130} = 13.22$, $p < 0.001$; year of hatching: $F_{19,130} = 0.35$, $p = 0.99$; interaction term: $F_{2,128} = 0.72$, $p = 0.49$). However, in our sample of adults, age and year of birth are strongly correlated ($r_S = -0.98$, $n = 63$, $p < 0.001$), making it difficult to separate effects of the two. When only adults were

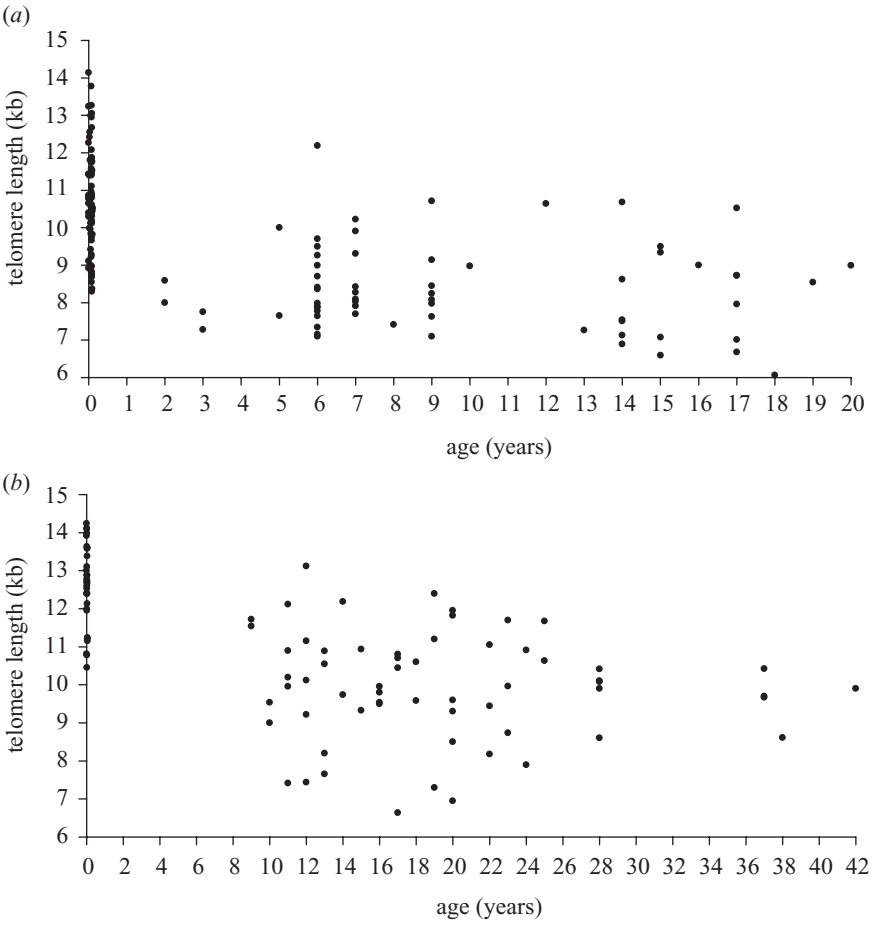

Figure 1. Cross-sectional comparisons of telomere length in (*a*) the European shag and (*b*) the wandering albatross. Telomere length appears to decline with age when all age classes are included. Reduced major axis regressions shag: $b = -0.30 \pm 0.02$ (s.d.) kb yr^{-1}, intercept = 10.96 ± 0.27 (s.d.) kb, $r = -0.59$, $n = 144$, $p < 0.001$; albatross: $b = -0.16 \pm 0.01$ (s.d.) kb yr^{-1}, intercept = 12.94 ± 0.38 (s.d.) kb, $r = -0.60$, $n = 92$, $p < 0.001$. However, among adults there is no significant relationship between telomere length and age (shags: $r = -0.08$, $n = 63$, $p = 0.54$; albatrosses: $r = -0.08$, $n = 61$, $p = 0.52$).

considered, hatching year did not explain a significant proportion of the variation in telomere length, nor did adult age become significant when year of birth was included (GLM both terms included, hatching year: $F_{17,44} = 0.20$, $p > 0.90$; age: $F_{1,44} = 0.03$, $p = 0.87$; interaction term: $F_{4,40} = 1.35$, $p = 0.27$). Neither within adults nor within chicks did the PCA score for natal environmental conditions relate to telomere length ($p > 0.05$). There was thus no evidence of cohort differences.

(b) *Longitudinal analysis of telomere length in shags*

In 15 out of the 16 shags sampled as chicks and again as adults, telomeres shortened (figure 2*a*; table 1). Although the general pattern was clearly for telomere length to decline between chick and adulthood (mean rate of loss -392 bp yr^{-1}), there was substantial variation in telomere length as a chick (coefficient of variation (CV) = 13%), the amount (CV = 59%) and percentage (CV = 55%) of telomere change, and in the estimated annual rate of change within individuals (CV = 75%). As cross-sectional analysis showed no detectable change in telomere length among adults, and suggests that there is little change during this time, the older a bird is when sampled for the second time, the slower the annual loss rate will appear to be. For those birds that showed a decline in telomere length, apparent rate of loss per year did decrease with the number of years between samples ($r_S = -0.56$, $n = 15$, $p = 0.03$), although the spread of ages in our data is rather limited. Because annual rate of loss may be biased by age at the second measurement, we used the total difference between chick and adulthood to examine environmental effects on telomere loss.

Individuals with the longest telomeres as chicks showed greater telomere shortening. This was true both in terms of absolute change (figure 2*b*; $r = 0.58$, $n = 16$, $p < 0.02$) and percentage change ($r = 0.50$, $n = 16$, $p < 0.05$). Taking this into account in a multivariate analysis, two other variables also explained substantial amounts of variation in telomere change. Greater telomere attrition was displayed by chicks laying down high tissue mass in relation to structural size (figure 2*c*), and by chicks from eggs laid relatively late in the season (figure 2*d*).

4. DISCUSSION

In shags and albatrosses, cross-sectional analysis suggests that a significant reduction occurs in blood cell telomere length between chicks and breeding adults; this decline was confirmed by longitudinal analysis in individual shags. However, no age-related telomere shortening was found among adults, sampled at a wide range of ages,

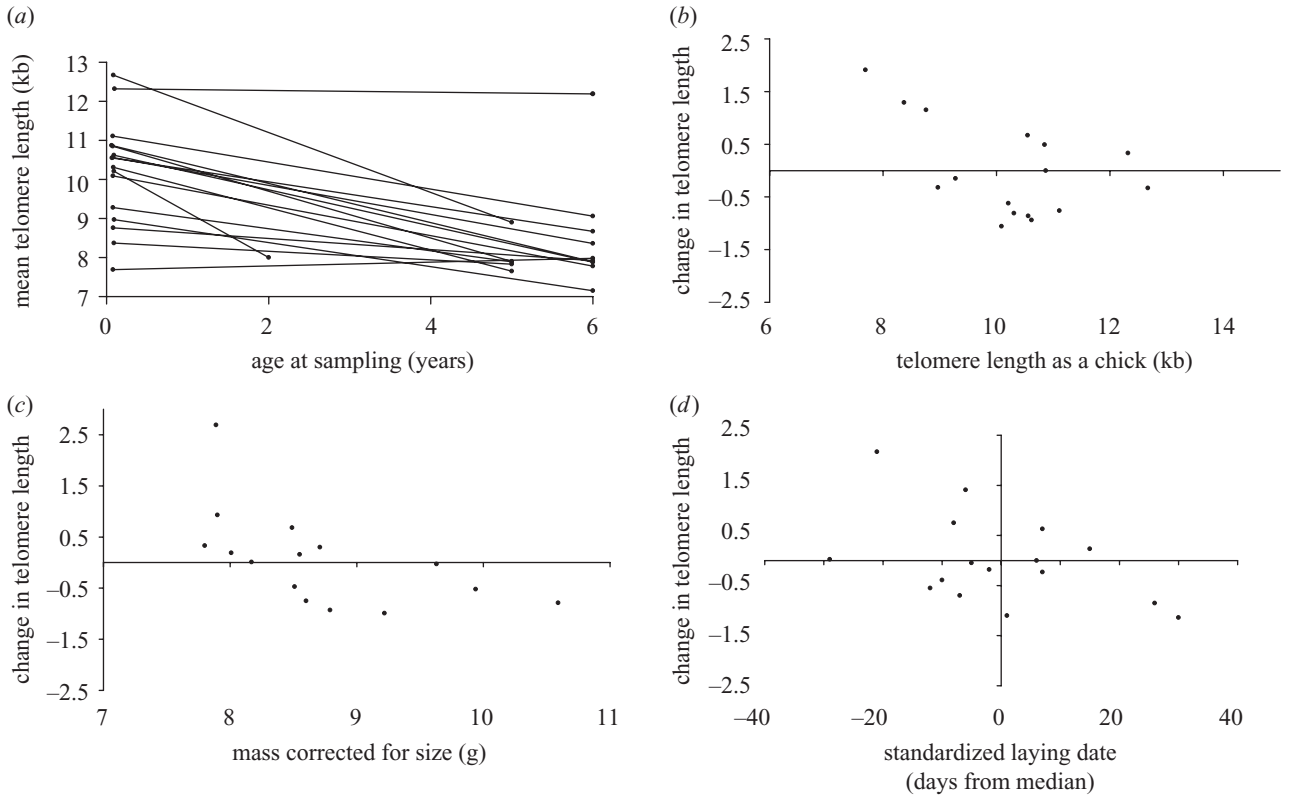

Figure 2. (*a*) Changes in the average length of telomeres in individual European shags with age. Lines link measurements of the same individual. Telomere length declined within individuals between chick and adulthood (paired *t*-test: $t_{15} = 6.8$, $p < 0.001$). Telomere length as a chick or adult was not related to mass, skeletal size or sex ($p > 0.05$). In a GLM analysis: (*b*) telomere length as a chick ($F_{1,11} = 7.36$, $p = 0.020$); (*c*) body mass corrected for size ($F_{1,11} = 12.31$, $p = 0.005$); and (*d*) the date on which eggs were laid, standardized for the median laying date in each year ($F_{1,11} = 6.54$, $p = 0.027$), all had independent and significant effects on the amount of telomere change between chick and adult life. (*b–d*) show standardized residuals for telomere change on the *y*-axes, having corrected for the other significant effects. Mass or skeletal size as an adult or chick, and sex had no effect on telomere change, and there were no significant interactions ($p > 0.05$).

Table 1. Telomere changes within 16 shags sampled once as a chick and for a second time in adulthood.

bird	age when sampled as a chick (days)	age when sampled as an adult (years)	telomere length as a chick (kb)	telomere length as an adult (kb)	difference in telomere length, adult–chick (bp)	rate of telomere change, adult–chick/time (bp yr^{-1})
1	29	6	7.69	7.98	+290	+49
2	36	6	12.32	12.19	−130	−22
3	33	5	8.37	7.83	−540	−110
4	30	6	8.76	7.92	−840	−142
5	29	6	9.28	7.89	−1390	−282
6	34	6	8.97	7.15	−1820	−308
7	25	6	10.55	8.67	−1880	−317
8	28	6	11.11	9.06	−2050	−346
9	29	6	10.56	8.36	−2200	−372
10	33	2	10.21	8.00	−2210	−1157
11	29	6	10.09	7.78	−2310	−390
12	31	5	10.31	7.65	−2660	−541
13	33	6	10.62	7.88	−2740	−464
14	28	5	10.85	7.90	−2950	−599
15	23	6	10.87	7.89	−2980	−502
16	31	5	12.67	8.90	−3770	−767

in either species. Our data from shags do not reveal any effect of natal year on telomere length as an adult or chick, thus suggesting that cohort effects are not strong and are unlikely to obscure a pattern of telomere loss with age. No sex difference in telomere length or telomere length change was found, in contrast to humans (Jeanclos *et al.* 2000, but see Vaziri *et al.* 1993), mice (Coviello-McLaughlin & Prowse 1997) and rats (Cherif *et al.* 2003).

Although we observed the same broad trend of age-related telomere loss as reported for several other bird

species (Haussmann *et al.* 2003*b*), our data also indicate that shortening does not occur at a constant rate with age. That telomere length differed between chicks and adults, but showed no relationship with adult age, suggests that the most rapid shortening takes place in early life, as is the case in other vertebrates where this has been studied (see Frenck *et al.* 1998; Rufer *et al.* 1999; Zeichner *et al.* 1999; Friedrich *et al.* 2001; Brummendorf *et al.* 2002). This is supported by the apparently steep decline in telomere length observed for the shag sampled at 2 years of age in the longitudinal comparison, which may be a consequence of the relatively short time interval over which the loss has been calculated in comparison with the other adults. However, more data are needed on changes within individuals, because if mortality is higher for those with very rapid telomere loss, samples of older birds will be biased towards individuals with slow attrition.

Early environmental differences can have effects later in life (Desai & Hales 1997; Lindström 1999; Metcalfe & Monaghan 2001, 2003; Lummaa & Clutton-Brock 2002), and telomeres are potential mediators of such processes (Aviv *et al.* 2003). Longitudinal comparisons exposed substantial variation among individuals in telomere length as a chick, and even greater variation in the amount of telomere change. Such variability in telomere attrition has also been noted in cells in culture (Serra *et al.* 2003). This suggests that individuals differ in their exposure or response to telomere modulating factors such as oxidative damage. We identified three variables that together explain 61% of the variation in telomere change observed in shag blood cells: telomere length as a chick, mass in relation to skeletal size and a seasonal effect. Individuals with longer telomeres as chicks have greater subsequent loss. Although there is likely to be a greater chance of recording a decline with measurements of larger magnitude, recent studies suggest that more telomeric repeats are lost from longer arrays (Marcand *et al.* 1999; Karlseder *et al.* 2002; Baird *et al.* 2003). There was also significantly greater telomere loss in individuals produced relatively late in their season of birth. Such chicks are more likely to experience nutritional stress as a consequence of lower parental quality, and possibly also seasonal changes in food supply. In shags, young pairs, especially first-time breeders, breed later in the season, lay smaller eggs and have lower breeding success than older pairs (Coulson *et al.* 1969; Potts *et al.* 1980; Aebischer 1993; Aebischer *et al.* 1995; Daunt *et al.* 1999). Interestingly, post-fledging survival to breeding age of early-hatched shags is higher than that of late-hatched individuals (Harris *et al.* 1994). Chicks heavy for their size also showed greater telomere loss. Both poor nutrition and rapid tissue growth are associated with higher levels of oxidative stress, which has been linked to telomere shortening (Jennings *et al.* 2000).

Interspecific differences in telomere rate of change (TROC) have been examined in relation to species maximum lifespan in both birds and mammals (Haussmann *et al.* 2003*b*). For such purposes, TROC has been taken as the slope of the regression line for telomere length versus age in cross-sectional comparisons. Our data suggest that the rate of telomere shortening is variable through life and consequently TROC will depend on the particular age distribution in the sample being assessed. The interspecific relationship calculated in Haussmann *et al.* (2003*b*) predicts that albatrosses, even with a conservative estimate of maximum lifespan, will show telomere lengthening of over 300 bp yr^{-1}, which is not the case. It is likely that if a relationship exists between telomere loss rate and lifespan, it is not linear. However, the apparently very low rates of change in adult birds are extremely interesting. Indeed, Haussmann *et al.* (2003*b*) found an intriguing positive relationship between telomere length and age in cross-sectional data from Leach's storm petrel (*Oceanodroma leucorhoa*). Although longitudinal study is needed to rule out cohort effects or selective mortality in producing this pattern, the slow rates of change in adult birds suggest that more active telomere maintenance may occur in these long-lived animals, presumably without an increased risk of cell immortalization and tumour development.

We thank Scottish Natural Heritage for access to the Isle of May National Nature Reserve. Mike Harris, Linda Wilson and Jude Hamilton provided help with fieldwork on the Isle of May; Daffyd Roberts and Dirk Briggs assisted in the collection of albatross samples from Bird Island. The British Antarctic Survey provided logistical support. Thanks to Kate Orr and Aileen Adams for help in the laboratory. M.E.H. was funded by a NERC research studentship.

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