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ORIGINAL ARTICLE

Maternal telomere length inheritance in the king penguin

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Telomeres are emerging as a biomarker for ageing and survival, and are likely important in shaping life-history trade-offs. In particular, telomere length with which one starts in life has been linked to lifelong survival, suggesting that early telomere dynamics are somehow related to life-history trajectories. This result highlights the importance of determining the extent to which telomere length is inherited, as a crucial factor determining early life telomere length. Given the scarcity of species for which telomere length inheritance has been studied, it is pressing to assess the generality of telomere length inheritance patterns. Further, information on how this pattern changes over the course of growth in individuals living under natural conditions should provide some insight on the extent to which environmental constraints also shape telomere dynamics. To fill this gap partly, we followed telomere inheritance in a population of king penguins (*Aptenodytes patagonicus*). We tested for paternal and maternal influence on chick initial telomere length (10 days old after hatching), and how these relationships changed with chick age (at 70, 200 and 300 days old). Based on a correlative approach, offspring telomere length was positively associated with maternal telomere length early in life (at 10 days old). However, this relationship was not significant at older ages. These data suggest that telomere length in birds is maternally inherited. Nonetheless, the influence of environmental conditions during growth remained an important factor shaping telomere length, as the maternal link disappeared with chicks' age.
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INTRODUCTION

Telomeres are highly conserved non-coding repetitive DNA sequences capping the ends of chromosomes and preventing them from being recognized as double-stranded breaks. Once telomeres reach a lower critical length, cell division arrest and/or cell senescence occurs (Blackburn, 2001). The rate at which telomeres shorten, therefore, defines cellular and organismal ageing, and depends on the balance between proeroding factors (such as oxidative stress or rate of cell division) and antieroding factors (such as the enzyme telomerase, mainly in germinal and stem cells, that actively rebuilds lost telomeres; Blackburn, 2001). Telomere length is also partly regulated by the telomere-associated shelterin protein complex (De Lange, 2005), and has consequently previously been defined as a polygenic trait (Andrew *et al.*, 2006; Gatbonton *et al.*, 2006).

Currently, emphasis is placed upon characterizing the extent to which variation in telomere loss might reflect life-history trade-offs and be involved in the evolution of life histories (Monaghan and Haussmann, 2006). For instance, telomere length at the end of growth has been found to predict accurately individual life expectancy both in captivity (Heidinger *et al.*, 2012) and in the wild (Bize *et al.*, 2009). Then, telomeres may be important life-history markers determining how much can be invested in maintenance efforts over the course of life (Eisenberg, 2011), 'powerfully quantifying life's insults' (Blackburn and Epel, 2012), and constituting a potential proxy of future individual fitness in a large variety of species from humans to birds (Cawthon *et al.*, 2003; Heidinger *et al.*, 2012). Therefore, telomere dynamics might be potential indicators of individual quality and could provide an insight into ecology and evolutionary biology.

Only traits with interindividual variation are of importance in evolution and are shaped by natural selection. Therefore, transmission and heritability (i.e. genetic and environmental variability in inheritance, estimated as the genetically based variation of inheritance expressed by h^2) of morphologic (Voillemot *et al.*, 2012), physiologic and behavioural traits, as well as ultimate fitness, have been widely studied (Kruuk *et al.*, 2000). It remains then important to check inheritance of fitness-related traits, lifespan in our case and of the mechanisms susceptible to shape them. Telomere length is highly variable among age-matched individuals (a trait suggested to have an early-life origin; (Hall *et al.*, 2004). Given significant heritability, telomere length variability is likely to provide a matrix for natural selection to act upon. Indeed, if telomere length is related to intrinsic trade-offs, phenotypes able to cope with (prevent) telomere erosion, thus repairing time and stress insults, may be at a selective advantage. The idea that ageing mechanisms may be partly inherited is supported by recent results in lizards, showing that ROS production (a proerosion factor for telomeres) is inherited from mothers to offspring (Olsson *et al.*, 2008).

At present however, knowledge on telomere length inheritance patterns, telomere length heritability and on the effects of parental age on telomere length in animals remains scarce. Those data are mainly restricted to humans (Nawrot *et al.*, 2004; Nordfjäll *et al.*, 2005, 2009; Unryn *et al.*, 2005; Njajou *et al.*, 2007), with results from studies on

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monozygotic and dizygotic twins (Slagboom *et al.*, 1994; Graakjaer *et al.*, 2004) (see Table 1 for a review). Most of these studies support paternal inheritance of telomere length (Nordfjäll *et al.*, 2005, 2009; Njajou *et al.*, 2007), further revealing that paternal age is a strong determinant of offspring telomere length (Unryn *et al.*, 2005; De Meyer *et al.*, 2007; Kimura *et al.*, 2008; Eisenberg, 2011). Interestingly, in non-human species contrasting results reveal either bi-parental inheritance patterns, for example, male inheritance in lizards (Olsson *et al.*, 2011) or maternal inheritance in birds (females being ZW; Horn *et al.*, 2011). These studies report highly variable heritability estimates ranging from 0.18 to >1.

Determining telomere length inheritance pattern is an important step in understanding the putative role of telomeres in lifespan variability. Given the scarcity of species for which telomere length inheritance has been studied (see Table 1), it is pressing to assess the generality of inheritance patterns in a broad range of species, in particular in wild populations. Further, there is an urgent need to test to which extent telomere inheritance patterns are maintained over growth, which should provide some insight into how far environmental constraints blur our perception of telomere length inheritance.

Consequently, we provide a case study of telomere length inheritance patterns in wild king penguins (*A. patagonicus*), a species where breeding parents raise a single chick that can be sampled early in life and followed over an 11-month-long growth period. We have previously shown that over this extended growth period, environmental challenges such as a prolonged winter fast, may have a tremendous impact on chick telomere loss (Geiger *et al.*, 2012). Here, we test for paternal and maternal influences on chick starting telomere length, and investigate how these relationships change over time as chicks age. Our protocol should allow distinguishing between genetic and environmental influences shaping telomere length in this long-lived seabird.

MATERIALS AND METHODS

Study site and animal model

This study was conducted on king penguins (*A. patagonicus*), in the colony of 'La Grande Manchotière' (20 000 breeding pairs) Possession island, Crozet archipelago (Terres Australes Antarctiques Françaises) located 46°25′S; 51°52′E. Data were collected during two consecutive field seasons (2009 and 2010).

The king penguin is a long-lived, pelagic seabird with a unique life cycle. The entire annual breeding cycle of adults lasts 14–16 months (including moult; Weimerskirch *et al.*, 1992) during which parents alternate between attending the chick on land and foraging at sea. In the middle of their growth period, chicks face 3 months of sub-Antarctic winter by their own, with a reduced food supply by the parents. Consequently, the entire growth period of chicks spreads over 11 months before they finally moult into subadult plumage and depart to sea (Weimerskirch *et al.*, 1992).

Table 1 Summary of the studies on telomere length inheritance patterns, telomere length heritability and link between parental age and telomere length

Species	*Father–offspring correlation*	*Mother–offspring correlation*	*Heritability*	*Outcome*	*Study*
Telomere length inheritance pattern					
Human (*Homo sapiens*)	Not measured	Not measured	$h^2=0.78$	Telomere length is genetically determined	Slagboom *et al.* (1994)
Human (*Homo sapiens*)	Not measured	Not measured	Not measured	Telomere length is genetically determined	Graakjaer *et al.* (2004)
Human (*Homo sapiens*)	Yes	Yes	Not measured	Telomere length inheritance linked to X chromosome	Nawrot *et al.* (2004)
Human (*Homo sapiens*)	Yes	No	Not measured	Paternal telomere inheritance	Nordfjall *et al.* (2005)
Human (*Homo sapiens*)	Yes	Yes	$h^2=0.44$	Paternal telomere inheritance	Njajou *et al.* (2007)
Human (*Homo sapiens*)	Yes	No	Not measured	Paternal telomere inheritance	Nordfjall *et al.* (2009)
Human (*Homo sapiens*)	Yes	Yes	$h^2=0.70$	Paternal and maternal inheritance, effect of paternal age	Broer *et al.* (2013)
Human (*Homo sapiens*) and	Not measured	Not measured	Not measured	Telomere length is inherited	Chiang *et al.* (2010)
Mouse (*Mus musculus*)	Not measured	Not measured	Not measured		
Kakapo (*Strigops habroptila*)	No	Yes	Not measured	Maternal inheritance of telomere length in a bird	Horn *et al.* (2011)
Telomere length heritability					
Human (*Homo sapiens*)	Not measured	Not measured	$h^2=0.82$		Jeanclos *et al.* (2000)
Human (*Homo sapiens*)	Not measured	Not measured	$h^2=0.34$		Bischoff *et al.* (2005)
Human (*Homo sapiens*)	Not measured	Not measured	$h^2=0.82$		Vasa-Nicotera *et al.* (2005)
Human (*Homo sapiens*)	Not measured	Not measured	$h^2=0.36$		Andrew *et al.* (2006)
Human (*Homo sapiens*)	Not measured	Not measured	$h^2=064$		Al-Attas *et al.* (2012)
Collared flycatcher (*Ficedula albicollis*)	Not measured	Not measured	$h^2=0.18$	Weak but significant telomere length heritability	Voillemot *et al.* (2012)
Parental age linked to telomere length					
Human (*Homo sapiens*)	Not measured	Not measured	Not measured	Offspring telomere length linked to paternal age	Unryn *et al.* (2005)
Human (*Homo sapiens*)	Not measured	Not measured	Not measured	Telomere length at birth linked to paternal age	De Meyer *et al.* (2007)
Human (*Homo sapiens*)	Not measured	Not measured	Not measured	Paternal age effects on offspring's telomere length	Kimura *et al.* (2008)
Human (*Homo sapiens*)	Not measured	Not measured	Not measured	Offspring telomere length linked to paternal age	Eisenberg *et al.* (2012)
Sand lizard (*Lacerta agilis*)	Not measured	Not measured	$h^2=0.52$/ $h^2=1.23$	Paternal age correlated to offspring's telomere length	Olsson *et al.* (2011)

General procedures

We followed 53 breeding pairs and started chick monitoring as close as possible to hatching, that is, 10 days afterwards to avoid breeding failure. Chicks were individually identified with fishtags and followed from hatching to the beginning of the final moult.

Chick telomere length dynamics was studied from blood samples collected from the marginal flipper vein at 10 (number of chicks = 53), 70 ($n = 37$), 200 ($n = 34$) and 300 days old ($n = 30$). During blood sampling, the chick's head was always covered with a hood to reduce its stress. Our sample size decreased over time owing to the fact that not all chicks survived the growth period (causes of death, likely predation, were not determined but were unrelated to handling). To investigate a potential link between parent and chick telomere length, brooding fathers were sampled when the chicks were 10 days old. Mothers were sampled at the next brooding shift, when they returned to the colony to relieve their partner some 15 days later (Descamps *et al.*, 2002). All blood samples were stored at −80 °C until analyses.

Telomere length measurements

Telomere length measurements were carried out when the chicks were 10, 70, 200 and 300 days. Telomere length was measured in DNA extracted from red blood cells (stored at −80 °C until analysis), which are nucleated in birds) using a Nucleospin Blood QuickPure Kit (Macherey-Nagel, Düren Germany). Telomere length was assessed by quantitative real-time amplification (qPCR) (Cawthon, 2002), a procedure previously described in birds (Bize *et al.*, 2009; Criscuolo *et al.*, 2009), including in king penguins (Geiger *et al.*, 2012). Relative telomere length is expressed as the (T/S) ratio of telomere repeat copy number (T) to a control single copy number gene (S). We used the *A. patagonicus* zinc-finger protein as a single control gene. Forward and reverse primers for the zinc-finger protein gene were 5′-TACATGTGCCATGG TTTTGC-3′ and 5′-AAGTGCTGCTCCCAAAGAAG-3′, respectively. Telomere primers were: Tel1b (5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGT TTGGGTT-3′) and Tel2b (5′-GGCTTGCCTTACCCTTACCCTTACCCTTAC CCTTACCCT-3′). qPCR for both telomere and the zinc-finger protein was performed using 2.5 ng of DNA with sets of primers Tel1b/Tel2b (or zinc-finger protein-F/zinc-finger protein-R), in a final volume of 10 µl containing 5 µl of BRYT Green fluorescent dye (GoTaqqPCR Master Mix; Promega, Charbonnieres les Bains, France). Primer concentrations in the final mix were 200 nM for telomere length determination and 300 nM for the control gene. Telomere and control gene PCR conditions were: 2 min at 95 °C followed by 40 cycles of 15 s at 95 °C, 30 s at 56 °C, 30 s at 72 °C and 60 s at 95 °C. Telomere and control (i.e. non-variable in copy number) gene amplification of each sample were carried out on the same plate. Each plate included serial dilutions (telomere and control gene, 5, 2.5, 1.25 and 0.625 ng) of DNA of the same reference bird which were run in triplicate. These serial dilutions were used to generate a reference curve on each plate, to control for the amplifying efficiency of the qPCR (both telomere and control gene). Mean amplification efficiency calculated from the reference curves of the qPCR runs were comprised between 100.9 and 103.1 (telomere) and 100.6 and 102.9 (control gene). R^2 calculated from the reference curves of the qPCR runs were comprised between 0.96–0.99 (telomere) and 0.97–0.99 (control gene). Intraplate mean coefficients of variation for Ct values were 1.35 ± 0.06% (telomere assay) and 0.79 ± 0.04% (control gene assay). Interplate coefficients of variation based on repeated samples were 1.56% (telomere assay) and 1.35% for (control gene assay). Mean coefficient of variation for the relative T/S ratios was 16%. To take into account the slight variation of efficiencies (E) between telomere and control gene amplifications, we calculated relative telomere length as: relative T/S ratios = ((1 + E telomere)^ΔCt telomere (control − sample)/(1 + E control gene)^ΔCt control gene (control − sample)) (see Pfaffl, 2001). Both a negative control (water) and melting curves were run for each plate to check for nonspecific amplification and primer–dimer artefacts.

Sex determination was carried out using DNA extracted from red blood cells. The method was adapted from (Griffiths *et al.*, 1998): 80 ng of DNA in a final volume of 20 µl, the PCR conditions were 2 min at 94 °C, followed by 10 cycles of a touchdown protocol: 30 s at 94 °C, 30 s at 60 °C (reducing the temperature of this step by 1 °C at each cycle), 35 s at 72 °C, followed by 30 cycles of 30 s at 50 °C and 35 s at 72 °C, finished by 5 min at 72 °C.

Statistical analysis

Telomere length (dependent variable) was log transformed to achieve a normal distribution and homoscedasticity. We assessed the time effect on telomere length using a generalized estimating equation (GEE). The time period (which corresponds to the different times of sampling during the growth period: 10, 70, 200 and 300 days posthatching) was entered as a repeated variable in the model, chick identity as a random factor, and chick sex, year of sampling, maternal and paternal telomere length as independent variables. All interactions between the different variables were tested. Nonsignificant interactions were removed sequentially from the analysis, starting with the least significant.

Then, to assess whether mother–offspring and father–offspring regressions were significantly different, we used a GEE with chick telomere length as the dependent variable. As before, time period was entered (10, 70, 200 and 300 days) as a repeated variable, chick identity as a random factor and parents' sex, telomere length and the interaction between parent's sex and telomere length as independent variables in the model

Finally, even though the interactions between parent's telomere lengths and the time period were not significant, we further tested for a potential link between chicks' telomere length and parents' telomere lengths over time by conducting a more exploratory analysis using Pearson's correlations. This link was tested at the four different chick ages.

The slopes of the regressions between both mid-parental and offspring telomere lengths, and between maternal and offspring telomere lengths were used to estimate narrow sense heritability: h^2 (the proportion of the phenotypic variance that is explained by additive genetic variance). To assess whether the mother–offspring h^2 was significantly different from the father–offspring one, we used a GEE with offspring telomere length as a dependent variable, chick identity as a random factor, parents' sex, telomere length and the interaction between parent's sex and telomere length as independent variables in the model.

All statistical analyses were performed using SPSS v. 18.0 (SPSS Inc., Chicago, IL, USA)). Significance level was set at 5%.

RESULTS

We found no sex or year effect on chick telomere length (Table 2, $P = 0.57$ and 0.82, respectively). However, there was a significant effect of the time period on offspring telomere length (Figure 1 and Table 2, $P < 0.001$), suggesting that telomeres were progressively eroded over the growth period (means values ± s.e.: 10 days (0.107 ± 0.02); 70 days (0.064 ± 0.024); 200 days (−0.06 ± 0.025); 300 days (0.012 ± 0.026)). In contrast to paternal telomere length, maternal telomere length was significantly related with chick telomere length (Table 2, $P = 0.028$).

Chick telomere lengths at 10 days were significantly correlated to maternal telomere length (Figure 2 and Table 3, $P = 0.042$), but not to paternal telomere length (Figure 3 and Table 3, $P = 0.388$). However,

Table 2 Results of the GEE model on offspring telomere length with time period as a repeated continuous variable, chick as a random factor and sex, year, maternal and paternal telomere length as independent variables in the model

Variable	*Wald* χ^2	P-*value*
Telomere length		
Year	0.050	0.823
Sex	0.318	0.573
Time period	29 317	0.001
Paternal telomere length	0.009	0.923
Maternal telomere length	4838	0.028

Abbreviation: GEE, generalized estimating equation.
The complete GEE model is presented after removing nonsignificant interactions. Further dropping remaining nonsignificant, single effects from the model did not change the significance of the maternal telomere length (Wald $\chi^2 = 5.585$, $P = 0.018$) nor the time period (Wald $\chi^2 = 32.723$, $P < 0.001$).

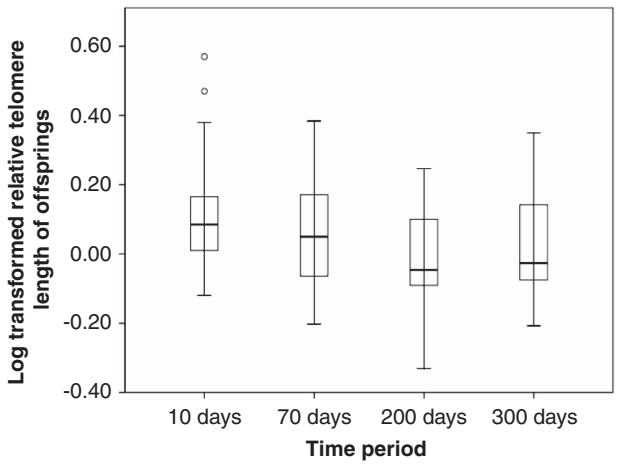

Figure 1 Log-transformed offspring telomere lengths over the growth period (10, 70, 200 and 300 days) (means values ± s.e.).

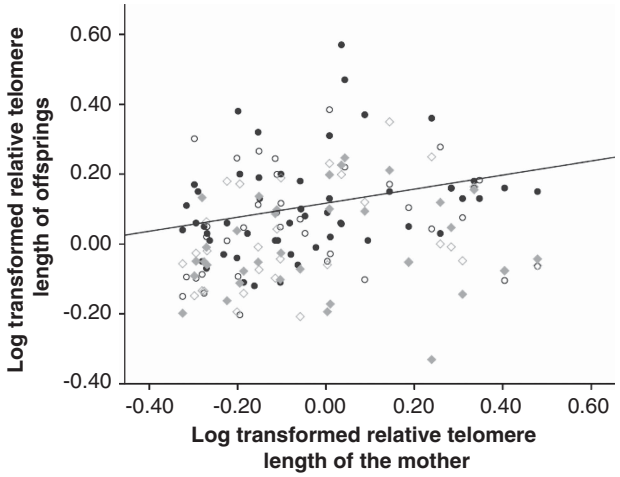

Figure 2 Mother–offspring correlations of log-transformed telomere length at 10 days (black filled circles), 70 days (black open circles), 200 days (grey filled diamonds) and 300 days (grey open diamonds). The black slope represents the significant mother–offspring correlation at 10 days.

the homogeneity of slope test showed no significant effect of the interaction between parent sex and parent telomere length (Table 4, $P = 0.513$), indicating that the mother–offspring and father–offspring slopes were not significantly different.

Heritability calculated using the slope of the regression between offspring and mean parents' telomere lengths was of 0.2 (s.e 0.110), and was of 0.2 (s.e. 0.096) for the regression between offspring and maternal telomere lengths. The homogeneity of slope test used to assess whether mother–offspring and father–offspring h^2 were significantly different showed no significant effect of the interaction between parent sex and parent telomere length (Wald $\chi^2 = 0.875$, $P = 0.350$). This indicates that the mother–offspring and father–offspring h^2 were not significantly different.

Offspring telomere lengths at 70, 200 and 300 days were not significantly correlated to maternal or paternal telomere length (Table 3).

Table 3 Pearson's correlations between offspring telomere length over the growth period and parental telomere length

	Paternal telomere length	*Maternal telomere length*
Telomere length 10 days (number of chicks = 53)		
r	0.122	0.284
P-value	0.388	0.042
Telomere length 70 days (n = *37)*		
r	0.027	0.198
P-value	0.873	0.240
Telomere length 200 days (n = *34)*		
r	−0.011	0.111
P-value	0.950	0.532
Telomere length 300 days (n = *30)*		
r	0.346	0.252
P-value	0.061	0.180

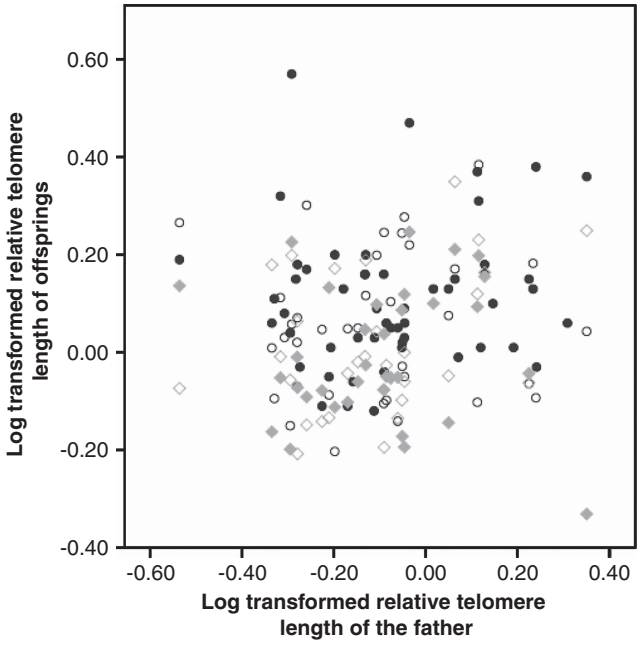

Figure 3 Father–offspring correlations of log-transformed telomere length at 10 days (black filled circles), 70 days (black open circles), 200 days (grey filled diamonds) and 300 days (grey open diamonds).

DISCUSSION

Our study shows that maternal and offspring telomere lengths are interrelated in the king penguin. However, our exploratory correlative analysis indicated that this link was displayed during early life (10 days) only, and found to become weaker (and nonsignificant) as chicks grew older. This result may be partly explained by selective mortality, chicks with short telomeres likely disappearing faster from the population (Geiger *et al.*, 2012). As these short telomere chicks were sampled only at 10 days, they may have produced enough variance in our sample to detect the significant correlation we observe at 10 days, but not later on. However, using only chicks that stayed alive for the entire experiment, the correlation remained significant at day 10 ($r^2 = 0.14$, $P = 0.038$, $n = 30$, mother–offspring h^2 of 0.2 (s.e. 0.1), midparental–offspring h^2 of 0.3 (s.e. 0.1)).

Moreover, maternal telomere length was linked to offspring telomere length, whereas paternal telomere length was not. Yet, we found no significant difference between father–offspring and

Table 4 Results of the homogeneity of slopes test using a GEE model on offspring telomere length with time period as a continuous repeated variable, chick identity as a random factor and parent sex, telomere length and the interaction between parent sex and telomere length as independent variables in the model

Variable	*Wald* χ^2	P-*value*
Telomere length		
Time period	29 671	0.000
Parents' sex	0.232	0.630
Parents' telomere length	3661	0.056
Parents' sex × parents' telomere length	0.428	0.513

Abbreviation: GEE, generalized estimating equation.
The complete GEE model is presented.

mother–offspring correlations. Therefore, paternal heritability of telomere length in this species might not be totally excluded, as it may have been masked by the difference in the age of sampling between chicks and their parents. Indeed, results might have been different had we sampled the parents when they were chicks.

Nonetheless, our findings are in accordance with previous data collected on the kakapo (Horn *et al.*, 2011), showing maternal inheritance of telomere length in birds. The pattern of telomere length inheritance displayed in birds seems to mirror the one found in several human studies (Nordfjäll *et al.*, 2005, 2009; Njajou *et al.*, 2007) and suggests that the heterogametic sex might have a key role in offspring telomere length determination.

Gene imprinting may be one of the mechanisms explaining why telomere inheritance is done through heterogamy. Imprinting occurs when both maternal and paternal alleles are present and one allele is expressed while the other remains silent (Pfeifer, 2000). Usually, the imprint marks distinguishing the parental alleles are epigenetic and generally due to DNA methylation and histone modifications, which can alter transcription patterns, and restrict gene expression (Pfeifer, 2000). To date, very few studies have identified imprinting of telomere length-regulating genes. Recently, Gao *et al.* (2011) identified a paternal imprinting on sperm chromatin that is essential for the inheritance of telomere identity in *Drosophila malanogaster.* Other studies determined that nucleotide polymorphisms occur in a large number of loci that code for proteins regulating DNA and histone methylation are associated with telomere length variability in humans (Gatbonton *et al.*, 2006; Blasco, 2007a). Therefore, a possibility would be that imprinting mechanisms might regulate expression of genes modulating telomere length (genes coding for telomerase and shelterin proteins), thus explaining the different patterns of telomere length inheritance observed. The regulation of telomere length by epigenetic factors (Blasco, 2007a) also supports the idea that imprinting mechanisms might be involved in telomere length inheritance. Indeed, histone modifications and DNA methylation seem to act as negative regulators of telomere length in humans/mice (García-Cao *et al.*, 2004; Gonzalo *et al.*, 2006). Epigenetic factors are known to impact directly telomere length, and also to regulate telomerase activity (Cong *et al.*, 2002). The *hTERT* gene promoter has been reported, as a rare exception, to be upregulated by methylation (Shin *et al.*, 2003; Nordfjäll *et al.*, 2005). Overall, it underlines that different pathways (from DNA and histone state to telomere maintenance protein expression) are likely to regulate telomere length inheritance (Blasco, 2007a).

Even though most studies conducted in humans suggest a paternal inheritance of telomere length (i.e. the mammalian heterogametic sex) (Nordfjäll *et al.*, 2005, 2009; Njajou *et al.*, 2007), some of them suggest that maternal inheritance also occurs (Nawrot *et al.*, 2004; Broer *et al.*, 2013; Eisenberg, 2013). As recently pointed out by Eisenberg (2013), the fact that some studies point to stronger father–offspring correlations, whereas others to stronger mother–offspring correlations is unlikely to be ascribed only to statistical noise, and at present no conclusion may be drawn.

These contrasting results indicate that the implication of the heterogametic sex is less straightforward than initially hypothesized. Indeed, the heterogametic pattern of telomere length inheritance observed in most studies might also be the result of confounding factors such as parental effects. Alternatively, it might be due to the fact that the link with the non-heterogametic sex is too weak to be detected. Investigation of telomere length inheritance patterns in more species (in complement to mammals and birds) with different sex determination systems, such as haplodiploidy and environmental sex determination (i.e. temperature dependent), might help solve whether telomere length is determined by the heterogametic sex.

Besides telomere length inheritance, some studies cited in Table 1 consider with more depth telomere length heritability, attempting to disentangle additive, non-additive genetic and environmental variability in inheritance patterns. Studies in humans reported heritability estimates of telomere length between 0.44 and 0.78 (Slagboom *et al.*, 1994; Njajou *et al.*, 2007) and of 0.52–1.23 in other vertebrates such as the sand lizards (*Lacerta agilis*), with an interesting difference in sex-specific heritability (Olsson *et al.*, 2011; see Table 1). However, these estimations were carried out mainly using mid-parent–offspring regressions (or comparisons between relatives) that may yield high heritability values (Conner and Hartl, 2004). Data on humans have led to the conclusion that individual variation in telomere length mainly originates from differences in the zygote and that epigenetic/environmental influences are relatively weak (Graakjaer *et al.*, 2004).

Nonetheless, a powerful approach to untangle genetic vs environmental effects is to use animal models with known genealogies or necessitates a cross-fostering design (Voillemot *et al.*, 2012). Chick exchange between nests was recently used in a wild population of collared flycatchers (Voillemot *et al.*, 2012). Heritability of telomere length (the proportion of the phenotypic variance explained by additive genetic variance) was found to be 0.18 (and not 9% as reported previously; P Bize, personal communication), a value close to the one we found here in king penguins (maternal estimate of $h^2 = 0.2$; mid-parent estimate of $h^2 = 0.2$), and supports the idea that additive-genetic effect is low in these two species. There are alternative explanations of these low heritability values. The first is that environmental factors are important determinants of early-life telomere length (Monaghan and Haussmann, 2006). Indeed, we found that the link between offspring and maternal telomere length early in life (10 days) was not maintained at a significant level over the growth period. Even if this result must be taken with caution as based on correlations established from a decreasing sample size with age, it partly supports that environmental factors have a strong influence on telomere loss in king penguin chicks (see Voillemot *et al.*, 2012). Further, Geiger *et al.* (2012) showed that faster-growing king penguin chicks loose telomere at a higher rate, illustrating the potential importance of early growth conditions on telomere dynamics. Such effects may have masked the link between offspring and maternal telomere lengths as chicks aged. Second, because telomere length is related to individual fitness (Bauch *et al.*, 2013), it is possible that natural selection has tended to deplete genetic variation of the loci-regulating telomere length in early life, thereby depleting the additive-genetic effect (Mirabello *et al.*, 2012). Finally, telomeres have been defined as a polygenic trait (Andrew *et al.*, 2006; Gatbonton *et al.*,

2006). As there are many more interactions possible between a greater number of loci (Lynch and Walsh, 1998), a third possibility could be that the non-additive genetic component of residual variance is large, thereby decreasing heritability but not because of additive-genetic variability depletion (Merilä and Sheldon, 1999). We could hypothesize that the pattern of telomere length heredity is dependent on the inheritance pattern of multiple telomere length restoration/maintenance factors (Blackburn, 2001; Blasco, 2007b). A resetting mechanism of early-life telomere length has been pointed out in mammals, in which both telomerase activity and parent telomere length are interacting to set-up the embryo starting telomere length (Chiang *et al.*, 2010). Focusing on these processes in the future might enable us to better understand how progressive telomere shortening over generations is avoided and how interspecific variability in telomere dynamics may have evolved.

Owing to the absence of siblings in our study, testing these alternatives was not possible. Still, a longitudinal following of the same breeders over years might enable us to estimate the additive and non-additive genetic components of transmitted telomere length (i.e. relative telomeres comparisons). Even more interesting is the fact that, due to the long development duration of their chick, king penguin alternatively breeds early or late in the season, depending on the fact that they were successful the preceding year (Weimerskirch *et al.*, 1992). Late breeders likely reproduce in a more stressful ecologic environment, because of a higher density of breeders later in the season (Viblanc *et al.*, 2011, 2014). Comparisons of the same pairs, during successive early and late breeding attempts, may enable us to estimate components of residual variance in telomere length such as ecological inheritance.

In conclusion, our study confirms that telomere length is maternally inherited in birds, supporting the hypothesis of inheritance from the heterogametic sex, and suggests strong environmental effects on telomere dynamics, as this link disappeared over time. However, in view of the present knowledge (Table 1), the results on telomere length inheritance and heritability patterns are still contrasted. For a better understanding of telomere length transmission, future works should focus on the whole balance (i.e. pro- and antierosion factors and their inheritance) that is going to shape the offspring telomere length, to understand accurately how telomeres are transmitted over generations. State may be as important as length in this case.

DATA ARCHIVING

Data available from the Dryad Digital Repository: doi:10.5061/dryad.4407g.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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