Molecular Ecology
August 2020, Volume 29 Issue 16 Pages 3155-3167
https://doi.org/10.1111/mec.15485
https://archimer.ifremer.fr/doc/00636/74773/

Foster rather than biological parental telomere length predicts offspring survival and telomere length in king penguins

Viblanc Vincent A. [1, 2, *] , Schull Quentin [1, 3], Stier Antoine [4], Durand Laureline [1, 5], Lefol Emilie [1, 5], Robin Jean-Patrice [1], Zahn Sandrine [1], Bize Pierre [6], Criscuolo François [1, *]

[1] Institut Pluridisciplinaire Hubert Curien UMR 7178 Université de Strasbourg CNRS, Strasbourg, France
[2] Centre d'Ecologie Fonctionnelle et Evolutive UMR 5175, IRD, CNRS, EPHE Université de Montpellier, Université Paul Valéry Montpellier 3, Montpellier, France
[3] MARBEC IFREMER, IRD CNRS Université de Montpellier, Sète, France
[4] Department of Biology University of Turku, Turku, Finland
[5] IPEV , Institut Polaire Français Paul Emile Victor, Plouzané, France
[6] School of Biological Sciences University of Aberdeen, Aberdeen, UK

* Corresponding authors : Vincent A. Viblanc, email address : vincent.viblanc@iphc.cnrs.fr ; François Criscuolo, email address : francois.criscuolo@iphc.cnrs.fr

Abstract :

Because telomere length and dynamics relate to individual growth, reproductive investment and survival, telomeres have emerged as possible markers of individual quality. Here, we tested the hypothesis that, in species with parental care, parental telomere length can be a marker of parental quality that predicts offspring phenotype and survival. In king penguins (Aptenodytes patagonicus), we experimentally swapped the single egg of 66 breeding pairs just after egg laying to disentangle the contribution of prelaying parental quality (e.g., genetics, investment in the egg) and/or postlaying parental quality (e.g., incubation, postnatal feeding rate) on offspring growth, telomere length and survival. Parental quality was estimated through the joint effects of biological and foster parent telomere length on offspring traits, both soon after hatching (day 10) and at the end of the prewinter growth period (day 105). We expected that offspring traits would be mostly related to the telomere lengths (i.e., quality) of biological parents at day 10 and to the telomere lengths of foster parents at day 105. Results show that chick survival up to 10 days was negatively related to biological fathers' telomere length, whereas survival up to 105 days was positively related to foster fathers' telomere lengths. Chick growth was not related to either biological or foster parents' telomere length. Chick telomere length was positively related to foster mothers' telomere length at both 10 and 105 days. Overall, our study shows that, in a species with biparental care, parents' telomere length is foremost a proxy of postlaying parental care quality, supporting the "telomere – parental quality hypothesis."

Keywords : gene and early life environmental effects, growth, penguins, reproduction investment, telomere

1 | INTRODUCTION

Telomeres are repeated DNA sequences at the end of chromosomes that play a key role in maintaining genome integrity (Gomes, Shay, & Wright, 2010). Telomere length can shorten over time in response both to cell division and stressors (including environmental stressors, psychosocial stressors, or poor early life conditions) (Levy, Allsopp, Futcher, Greider, & Harley, 1992; Tomiyama et al. 2012; Boonekamp et al. 2014; Hanssen et al. 2017; Chatelin et al. 2019; Noguera et al. 2019; Saulnier et al. 2020; but see Cerchiara et al. 2017). As a consequence, telomere lengths and their dynamics have been related to individual health and stress at a proximate level (Verhulst et al., 2016) and to fitness-outcomes at various life history stages (Bauch et al. 2013; Bize et al. 2009; Heidinger et al., 2012; Salomons et al., 2009). Therefore, telomeres are increasingly considered as a cellular proxy of multiple correlated phenotypic traits that define individual quality (Angelier el al., 2019). This 'telomere - individual quality hypothesis' predicts that individuals with longer telomeres may benefit from both higher survival and reproductive rates (Angelier el al., 2019). For species with parental care, an extrapolation of this 'telomere - individual quality hypothesis' is that parental telomere length may reflect parental quality, parents with longer telomeres being better at raising a large number of high quality offspring with high survival rates (i.e. 'telomere - parental quality hypothesis'). Remarkably, because telomeres are genetic material passed on from parents to offspring, one topical question is also to which extent parent-offspring resemblance in telomere length is explained by genetic additive variance (heritability) and/or by other environmental effects caused by variation in the quality of pre- and post-hatching parental care (Belmaker et al. 2019).

Early studies suggested that telomere length is fixed in the zygote (*i.e.* inherited from the gametes in a sex- and age-dependent way; Eisenberg, 2019), remaining unchanged for life relative to others individuals from the same cohort (Graakjaer et al., 2004). However,

64 estimates of telomere length heritability appear to be largely variable across species (Asghar
65 et al. 2014; Atema et al., 2015; Becker et al., 2015; Stier et al., 2015; Belmaker et al., 2019),
66 suggesting that both genetics and environmental factors (including parental care) may
67 influence offspring telomere length. Assessing the effects of parental care quality on telomere
68 length is however complex, since it requires disentangling the contribution of additive genetic
69 effects (i.e. heritability) from parental care *per se* on offspring telomeres. In fact, individual
70 telomere length within its cohort appears not to be fully established at the embryonic stage but
71 changes rapidly in early-life (Fairlie et al., 2016), mostly during growth when cell division
72 rates are high (Monaghan & Ozanne, 2018). A large number of non-exclusive mechanisms
73 can account for inter-individual variability in telomere length early in life. In birds for
74 instance, telomere length may vary according to embryo exposure to maternal corticosterone
75 in the egg (Haussman et al. 2012), incubation temperature (Stier et al., 2019), and/or variation
76 in post-hatching environmental conditions (Nettle et al., 2015; Reichert et al., 2015; Soler et
77 al., 2017). Those post-hatching factors include the quality of parental care and/or parental
78 effort (as suggested by positive links between parental telomere length and breeding
79 performance; Le Vaillant et al., 2015; Angelier et al., 2019, but see Bauch et al., 2013; Young
80 et al., 2016). In this context, the use of cross-fostering designs combined with longitudinal
81 measurements of offspring growth trajectories, telomere length dynamics, and survival
82 (Boonekamp et al. 2014; Bauch, et al. 2019; Criscuolo et al., 2017; Dugdale & Richardson,
83 2018; McCarty, 2017) may prove particularly powerful in gaining new insights on the
84 proximate genetic and post-laying environmental determinants of telomere length variability
85 in the next generation.

86 We applied such an approach to study the growth trajectories and telomere length
87 dynamics of king penguin chicks (*Aptenodytes patagonicus*) during the first 3 months of their
88 development. King penguins are slow-breeding seabirds where bi-parental care is required to

89 successfully rear a single chick over a 14-month period. Parental quality is therefore of critical
90 importance in this species (Stonehouse 1960). In this study, we exchanged eggs between
91 breeding pairs soon after egg laying, and we measured both adult telomere length shortly after
92 mating and their chick phenotype at 10 and 105 days after hatching (shortly after hatching and
93 towards the end of their pre-winter growth period, respectively). During the winter period,
94 chicks gather into "crèches" with almost no parental care (Stonehouse 1960; Geiger et al.,
95 2012; Saraux et al., 2012). Hence, this experimental cross-fostering design allowed us to
96 disentangle the contribution of biological (mostly investment in eggs and genetics) *vs.* foster
97 (mostly incubation and chick rearing) parental quality assessed as parental telomere length
98 (i.e. telomere length is positively associated with breeding success in adults; Le Vaillant et al.,
99 2015) on chick structural size, body condition, telomere length and survival in early life (i.e.
100 at 10 and 105 days). In the king penguin, chick body condition and telomere length soon after
101 hatching (day 10) are good predictors of survival (Geiger et al., 2012; Stier et al., 2014).
102 Telomere length also shortens with age during chick growth (Geiger et al., 2012; Stier et al.,
103 2014), but does not appear to be related with age in adults (aged 5 to 9 years old; Le Vaillant
104 et al., 2015). If chick phenotype and telomere length soon after hatching are mostly
105 determined through genetic and/or early maternal effects (i.e. investment in eggs), we
106 expected chick phenotypes, including chick telomere length, to be positively related to the
107 telomere lengths of their biological parents. However, because post-laying parental quality,
108 measured through telomere length of foster parents, is likely to become apparent as chicks
109 grow and receive increasing amounts of parental care, we predicted foster parental telomere
110 lengths to be positively related to chick structural size, body condition and survival at 105
111 days. Telomere inheritance was previously found to be moderate ($h^2 \sim 0.2$), being stronger
112 early in development (day 10 after hatching) and fading during development (up to day 300
113 after hatching) in this species (Reichert et al., 2015). Hence, in this study we also tested

whether the resemblance between biological parent and offspring telomere length (i.e. genetic effects) diminished during offspring development and was replaced by post-hatching environmental influences measured through a positive resemblance between foster parent-offspring telomeres, as offspring aged.

When investigating the effects of parental quality on offspring phenotype and survival, it is essential to keep in mind that parental quality typically increases with age as individuals gain experience over successive breeding seasons (Forslund & Pärt, 1995; Lecomte et al., 2010). Interestingly, the 'age – parental quality' and 'telomere – parental quality' hypotheses lead to opposite predictions. On one hand the 'age – parental quality hypothesis' predicts that older parents should be of higher quality. On the other hand, older parents are expected to have shorter telomeres and therefore to be of lower quality according to the 'telomere – parental quality hypothesis'.

2 | MATERIAL AND METHODS

2.1 | Study species and breeding pair monitoring

This study was conducted in the king penguin colony of "La Baie du Marin" (Possession Island, Crozet Archipelago, 46°26' S – 51°52'E), home to some 24,000 pairs of breeding birds. In 2012-2013, we monitored 66 breeding pairs of unknown age from courtship (early November) up to the onset of the Austral winter (early April). In king penguins, the breeding cycle is long and complex, starting by a courtship period of ~15 days during which pairs will form, select a breeding territory, and females lay their single egg (Stonehouse, 1960). Following egg-laying, males and females alternate between periods on land, incubating the egg or caring for the chick, and periods foraging at sea for the rest of the summer (Weimerskirch et al., 1992). The female is the first to leave for sea, the male taking charge of the first incubation shift (Weimerskirch et al., 1992). Incubation lasts for ~53 days

(Stonehouse, 1960), the egg typically hatching during incubation shift 4 (the female's second incubation shift). The chick's growth period extends over 10-11 months, including an energy-constraining winter period (April to September) during which it is seldom fed and loses substantial body weight (Cherel et al., 1985; Weimerskirch et al., 1992). Chick feeding and growth resume the following summer (Weimerskirch et al., 1992). Following chick fledging, parents have to moult and replenish their energy stores before they are ready for a subsequent breeding season (Weimerskirch et al., 1992). Divorce rates between breeding seasons are high (*ca.* 80%; Olsson, 1998), however, within a season cooperation between partners is critical to successfully raise the chick, *i.e.* a single parent can not succeed. Parental quality is key and mutual mate choice for high quality partners is high in this species (Jouventin & Dobson 2017).

We first marked both male and female pair members on the chest from a 1-m distance using animal spray dye (Porcimark®, Kruuse, Lageskov; Denmark) when they were settling on their final breeding territory. The pair was monitored daily at a distance, using binoculars, until a single bird was observed incubating the egg. This bird was identified as the male at day 1 of incubation and, 3 days after egg-laying (to minimize disturbance until the bird was motivated to incubate), was flipper-banded with semi-rigid PVC Darvic bands (25.8mm wide, 1.9mm thick, 7.4g), allowing its identification and subsequent monitoring during the study. The female was caught and flipper-banded when she returned from her first foraging trip at sea. All flipper-bands were removed from birds at the end of the study.

2.2 | Cross-fostering design, blood sampling and bird monitoring

Three days after the egg was laid (first incubation shift of the male), we cross-fostered (*i.e.* swapped) eggs between penguin pairs that had laid their egg on the same day. In total, we swapped eggs between 66 breeding pairs grouped in 33 dyads. This required 3 persons. First,

two males were immobilized while incubating in the colony and rapidly hooded to minimize stress. Their respective egg was carefully removed from the brood pouch and replaced by a warm dummy plaster egg during the exchange. Eggs were weighed to the nearest 1-g using a Pesola® spring-slide scale. One person then proceeded to exchange the eggs while the 2 other persons remained by the birds in the breeding colony at all times to ensure the procedure went smoothly. Once the eggs were swapped and individuals released, we monitored bird behaviour to ensure they settled down once again on their breeding territory. We never witnessed breeding abandonment by the birds at this stage.

Adult monitoring

For males and females, blood samples (2 mL) were collected from the marginal flipper vein using a G22-1½ needle fitted to a 2.5 mL heparinized syringe. Males were sampled at the time cross-fostering occurred (day 3, incubation shift 1). Females were sampled during their first incubation shift (day 2). The bird's head was covered with a hood to minimize stress and agitation during blood sampling, and samples were kept on crushed ice in the field until further processing, usually within 15 min. After centrifugation (3000g for 10 min), plasma and blood cells were separated and kept frozen dried at -20°C until the end of the day, before being moved to -80°C until assayed. Penguin pairs were monitored twice daily until hatching (confirmed by the presence of a newly hatched chick and the presence of broken egg shells). That day was marked as hatching day. Ten days later, we caught the adults as described above, and temporarily replaced the chick with a warm dummy plaster egg.

Chick monitoring

On day 10 post-hatching (*i.e.* early during development), chicks were measured for flipper length, beak length and tarsus length to closest 1-mm using a solid metal ruler. They were

weighed (closest 5g) using a spring-slide Pesola® scale, and a small blood sample (~100 µL) was obtained from the marginal flipper vein using a G27-1½ needle and 75 µL heparinized capillary tubes. Chicks were then individually identified using color-coded fish tags (Floy Tag and MFG, Inc. Seattle, WA, USA) attached subcutaneously to their upper-back (Stier et al., 2014). On day 105 post-hatching, the same procedure was repeated, when chicks had been emancipated for approximately two months and had gathered in crèches in anticipation of the austral winter period. We then collected 1 mL of blood from the marginal flipper vein, and measured flipper length, beak length and tarsus length as described above.

From these data, we calculated chick structural size as the first principal component of separate PCA analyses on flipper length, beak length and tarsus length both at 10 and 105 days (SSz_{10} = -28.44 + 0.29 beak + 0.12 flipper + 0.11 tarsus; SSz_{105} = -33.80 + 0.12 beak + 0.03 flipper + 0.08 tarsus; ΔSSz = -21.16 + 0.12 beak + 0.04 flipper + 0.08 tarsus). Because chick body mass and structural size indices were highly correlated (at day 10: Pearson's r = 0.87, t = 12.72, $df = 52$, $P < 0.0001$; at day 105: r = 0.76, t = 7.42, $df = 41$, $P < 0.0001$), we calculated chick body condition at day 10 and day 105 by regressing body mass on structural size at those different time points (Schulte-Hostedde et al. 2005). Chick structural size and body condition were then used as uncorrelated dependent variables in subsequent analyses (see below).

Chicks were monitored up until the subsequent summer (November-December), when they departed from the colony for their first trip at sea. Of the 66 eggs produced by the monitored breeding pairs 54 chicks survived up to 10 days and 44 chicks survived up to 105 days.

2.3 | Measurement of telomere length in adult and chick king penguins

King penguin relative telomere length (RTL) was measured using a protocol specifically developed and routinely used on king penguins (Geiger et al., 2012; Reichert et al., 2015; Le Vaillant et al., 2015; Stier et al., 2014; Schull et al., 2018). DNA was extracted from nucleated red blood cells (Nucleospin Blood QuickPure, Macherey-Nagel, Düren, Germany) and checked for quality using gel-migration and a NanoDrop 1000 (Thermo Scientific) spectrophotometer (absorbance ratio A260/280; A260/230.). Extracted DNA was then used to amplify both the telomere and a control gene (non-variable in copy numbers within our population, Smith, Turbill & Penn, 2011) by quantitative real-time amplification (qPCR) based on Cawthon's original development (Cawthon, 2002). Control gene (*Aptenodytes patagonicus* zinc finger) and primer sequences were identical to those used in previous penguin telomere studies, as well as the conditions of qPCR amplifications (see Stier *et al.*, 2014 for details). We used 2.5 ng DNA per reaction and the BRYT Green fluorescent probe (GoTaq_qPCR Master Mix; Promega, Charbonniere, France). The samples were amplified on a 384 wells thermocycler (CFX-384, Biorad Hercules), in duplicates over three runs, the telomere sequence and the control gene sequence being amplified using the same conditions. Samples were distributed over 3 plates and individual birds randomly distributed on each plate. Intra-plate repeatability based on duplicate runs was of 0.785 for the final calculated relative telomere length value (T/S ratio based on Cq values). Inter-plate repeatability based on 13 samples (*i.e.* 13 different individuals) repeated over all plates was of 0.894 for final calculated relative telomere length value (T/S ratio). Mean amplification efficiencies of telomere sequence and control gene were of 100% and 99.9% (plate 1), of 100.2% and 99.8% (plate 2) and of 100% and 100.3% (plate 3), respectively. Relative telomere lengths were calculated following (Pfaffl, 2001) and using the plate efficiencies amplification values corresponding specifically to each sample. No apparent well-position bias was observed

(Eisenberg, Kuzawa, & Hayes, 2015) (see Online Supporting Information). We obtained telomere data for 61 adult breeding pairs and 42 chicks throughout growth.

2.4 | Statistical analyses

All analyses were run using R v.3.5.1. Forest plots and marginal effects plot with 95% CI were obtained using the 'sjPlot' package in R (Lüdecke, 2017). In all models presented below, Relative Telomere Length (RTL) was systematically log-transformed and standardized (z scores) prior to analyses (see Verhulst et al. 2019). Other continuous variables were standardized so that model coefficients could be directly comparable in their magnitude. Where appropriate, we ensured residuals were normally distributed by visual inspection of density distributions, Q-Q plots, cumulative distribution functions and P-P plots using the 'fitdistrplus' package in R (Delignette-Muller & Dutang, 2015). We also ensured that no substantial collinearity occurred between independent variables (Variance Inflation Factors ranged $1.05 < VIF < 2.05$; suggested cut-off at 3; Zuur, Ieno, & Elphick, 2010). For each model, sample sizes are reported in the tables. Sample sizes can vary across models due to variation in egg and chick mortality and/or due to difficulties at sampling blood from some chicks or amplifying DNA (telomeres) from some blood samples.

Chick telomere dynamics during growth

We investigated chick RTL dynamics in early life using linear mixed models (LMMs) with RTL as the dependent variable, chick age (categorical: 10 or 105 days after hatching) as an independent variable, and chick ID as a random factor. Hence, the model was specified as:

$$z\text{-}RTL \sim Chick\ age_{10\ or\ 105} + (1|chick\ ID)$$

From this model, we computed repeatability in chick RTL during early life as the ratio of among-individual variance (V_G) over the total phenotypic variance (V_P) equal to $V_G + V_R$

(the within-individual or residual variance in RTL) (see Nakagawa & Schielzeth 2010; Stoffel et al. 2017). Hence, repeatability = $R = \frac{V_G}{V_P} = \frac{V_G}{V_G + V_R}$. This LMM-based repeatability estimate allowed to control for the confounding effects of age (see Nakagawa & Schielzeth 2010) since chick RTL shortened with time. Repeatability was calculated using the 'rptR' R cran package; Stoffel *et al.*, 2017). Confidence intervals around the repeatability estimate were computed by parametric bootstrapping (10,000 iterations). This repeatability allowed us to assess whether chicks starting their post-hatching growth period with long telomeres also entered the winter period with long telomeres, which informs on the importance of 'starting' telomere length in determining later life telomere length and potentially life histories.

Chick survival and phenotype in relation to parental RTL

Chick survival: We tested for influences of parental (both biological and foster) RTL on chick survival up to day 10, or up to day 105, using separate Generalized Linear Mixed Models (GLMMs: binomial, logit-link). These were specified as:

$$Survival\ (0 = failure,\ 1 = success)_{10\ (or\ 105)} \sim z\text{-}RTL_{biological♂} + z\text{-}RTL_{biological♀} + z\text{-}RTL_{foster♂} + z\text{-}RTL_{foster♀} + z\text{-}egg\ mass + (1|dyad)$$

Here, we included cross-fostering dyad identity as a random factor in the model and accounted for egg mass as a covariate to test for potential effects occurring from early maternal investments in the egg (Bize et al. 2002; Krist 2011). From these models, odd ratios were calculated to illustrate the relative influence of the different fixed factors (mainly in our case biological and foster parental telomere lengths) on offspring survival. The odd-ratio can be interpreted for a given predictor in terms of increasing (>1) or decreasing (<1) the likelihood to survive for a one unit increase in that predictor, holding all other variables constant. For instance, holding all other variables constant, an odds ratio of 2 for a given

predictor would imply that the odds of surviving increase by a factor 2 for each unit increase in the considered predictor.

Chick phenotype: The influence of biological and foster parent RTL on chick phenotypic traits (structural size, body condition and RTL) both early (10 days post-hatching) and later (105 days post-hatching) in life were tested using separate LMMs. Here also, we accounted for egg mass as a covariate in the models, and controlled for cross-fostering dyad identity as a random factor. These were thus specified as:

$$z\text{-}Phenotypic\ trait \sim z\text{-}RTL_{biological♂} + z\text{-}RTL_{biological♀} + z\text{-}RTL_{foster♂} + z\text{-}RTL_{foster♀} + z\text{-}egg\ mass + (1|dyad)$$

Finally, we tested the influences of both biological and foster parent RTL on the change in chick RTL between days 10 and 105 ($RTL_{105} - RTL_{10}$) using a Linear Mixed Model (LMM). We specifically chose not to control for chick initial telomere length in this model (RTL_{10}), since this may lead to biased estimated of rate of attrition even when correcting for regression to the mean (Bateson et al. 2019). We included cross-fostering dyad identity as a random factor in the model to account for potential temporal effects associated with the cross-fostering design (eggs being swapped on the same date between dyads of penguin pairs). The model was thus specified as:

$$z\text{-}(RTL_{105} - RTL_{10}) \sim z\text{-}RTL_{biological♂} + z\text{-}RTL_{biological♀} + z\text{-}RTL_{foster♂} + z\text{-}RTL_{foster♀} + (1|dyad)$$

3 | RESULTS

3.1 | Chick telomere dynamics in early life

On average, chick telomere length decreased over time (LMM; $z\ RTL_{105vs10}$ = -0.40 ± 0.14, t = -2.78, CI = [-0.68; -0.12], P = 0.008; Fig. 1). Using the variance explained by chick ID in this model (0.55 ± s.d. 0.74), we found that chick telomere length was repeatable (LMM; r = 0.56 ± 0.11, CI = [0.33; 0.74], P < 0.001): chicks starting their post-hatching growth period with longer telomeres also entered the winter period with longer telomeres (see Figs. 1a and 1b).

3.2 | Chick survival and phenotype at 10 days

Chick survival up to 10 days was weakly and negatively related to the RTL of the biological male, but not to the RTL of the biological mother, the RTL of foster parents, or egg mass (Table 1, Fig. 2a and 3a). At 10 days, neither chick structural size or body condition were significantly related to biological or foster parental RTL telomere length, or egg mass (Table 1, Figs. 2b and 2c). In contrast, chick RTL was positively associated with the RTL of foster mothers (Table 1, Figs. 2d and 3b), and positively (though not significantly, P = 0.071) with the RTL of foster fathers, but not with the RTL of genetic parents or egg mass (Table 1).

3.3 | Chick survival and phenotype at 105 days

At 105 days, chick survival was significantly and positively related to foster male RTL, but not to the RTL of the foster mother, the RTL of biological parents, or egg mass (Table 2, Fig. 4a and 5a). At 105 days, neither was chick's structural size or body condition significantly related to biological or foster parental RTL telomere length, or egg mass (Table 2, Figs. 4b and 4c). In contrast, chick RTL was significantly and positively associated with the RTL of foster mothers (Table 2, Fig. 4d and 5b). The change in chick telomere length between days 10 and 105 was not significantly associated with parental RTL when both biological and foster parents were included in the same model (Table 3).

4 | DISCUSSION

Using an experimental cross-fostering approach in the king penguin, our study aimed at identifying the contributions of pre-laying (genetics and egg mass) and post-laying (incubation, brooding and feeding) parental quality on offspring phenotype and survival. We hypothesised that parents with longer telomeres were of higher quality. We tested whether offspring phenotype either soon after hatching (day 10) or at the end of the pre-winter growth period (day 105) were best explained by pre-laying and post-laying parental quality measured via, respectively, the measures of telomere length of their biological and foster parents. Our results highlight an overall larger impact of foster parental RTL on chick survival over the growth period, as well as concomitant impact on chick RTL. This supports the idea that telomere length is a measure of parental quality that can (i) predict post-laying parental investment into their offspring and (ii) modulate next generation telomere length.

4.1 | Parental telomere length effects on chick survival

Because of their susceptibility to environmental stress, telomeres have been proposed as integrative markers that can be used to reflect an individual's life stress and by extension stress coping mechanisms, thus perhaps allowing to gauge individual quality (Angelier *et al.*, 2019). From an evolutionary perspective, high quality individuals are expected to perform well in a suite of correlated phenotypic traits, including investment in parental care (Wilson & Nussey, 2010). Hence, one of the aims of this study was to test the 'telomere – parental quality hypothesis' hypothesizing that parents with long telomeres were of higher quality, and therefore predicting that they should produce heavier and larger chicks more likely to survive early in life. Accordingly, previous studies have reported positive links between telomere length and reproductive success in seabirds, including king penguin (Angelier et al. 2019, Le

360 Vaillant et al. 2015; but see Olsson et al. 2011a for a quadratic association, and Bauch et al.
361 2013 for a negative association).

362 Surprisingly, after controlling for egg mass (*i.e.* maternal effects; Krist 2011), we
363 found a negative effect of biological father telomere length on chick survival at 10 days, but
364 no significant effect of foster parent telomere length (*i.e.* early post-hatching environmental
365 effects). Contrary to our expectation based on the 'telomere – parental quality hypothesis',
366 this result suggests that fathers with longer telomeres (expected to be of good quality)
367 somehow reduced the chances of survival of their chicks in the first days after hatching. This
368 negative effect was rather marginal (Table 3) and the mechanism explaining such an
369 association remains unclear. It seems unlikely this result was explained by the 'age – parental
370 quality hypothesis' (Forslund & Pärt, 1995; Lecomte et al., 2010), given a lack of association
371 between telomere length and chronological age in king penguins (Le Vaillant et al., 2015).
372 Furthermore, if father's age and experience were important determinants of chick survival in
373 penguins, we would have expected to detect a negative impact of foster father telomere length
374 on chick survival at 105 days.

375 In contrast, chick survival at 105 days increased with foster male telomere length,
376 even when controlling for egg mass. This is predicted by the 'telomere – parental quality
377 hypothesis' if indeed telomere length acts as a proxy of individual quality and positively
378 correlates with post-hatching paternal care. Interestingly, this effect was apparently
379 independent of any effect of paternal telomere length on chick body mass or growth,
380 suggesting other benefits than those purely related to energy investments in the offspring.
381 Telomere length has been positively associated to foraging efficiency, but not to parental
382 investment, in other seabird species (Young et al., 2015, 2016). In king penguins, if parental
383 foraging efficiency was also related to telomere length, we might expect parents with longer
384 telomeres to be better at provisioning their chicks during development, ultimately affecting

chick body mass or structural size. We found however no support for such mechanism. Remarkably, in king penguins on-land predation of brooded chicks is high (i.e. 51 % of crèching chicks in a given reproductive season; Descamps et al., 2005), and an important source of extrinsic mortality. Hence, an alternative mechanism could be that foster males with long telomeres are more territorial and aggressive birds and therefore better at coping with predators during their brooding shifts. This alternative mechanism remains to be tested.

4.2 | Parental telomere length effects on chick telomere length

Individual variation in telomere length in early life may come from (i) how zygote telomere length is determined and (ii) what inherited and environmental factors are going to change the way offspring lose and repair their telomeres. Disentangling those genetic and pre/post-laying influences is far from being an easy task because telomere length is a complex structure underpinned by the expression of multiple genes, by epigenetic modulation (Bauch et al., 2019), as well as by a wide number of environmental factors (Dugdale & Richardson, 2018). In addition, any modulation of development, of genetic (*i.e.* parental age, Bauch et al. 2019) or environmental origins (Metcalfe & Monaghan, 2003), may have pervasive impact on the future phenotype of offspring, including telomere length (Metcalfe & Monaghan, 2003; Tarry-Adkins et al., 2009). In this study, we swapped eggs soon after laying to investigate whether offspring telomere length were more alike the telomere length of their biological (genetic effect) or foster parents (pre/post-hatching parental effect).

Our results show that chick telomere lengths at 10 and 105 days were both related to foster maternal telomere length. At day 105, offspring telomere length was also positively related to biological mother and foster father telomere length, though not significantly. Previous data based on biological mother-offspring regressions have reported significant maternal heritability for telomere length in king penguin (around h^2 = 0.2), which weakened

410 over the period of chick growth (Reichert et al., 2015). Thus, although telomere length in king
411 penguin chicks may be determined in part before egg-laying (e.g. Olsson et al. 2011a; Bauch
412 et al., 2019), our data suggest a stronger effect of the post-laying environment on chick
413 telomere length (see Becker et al., 2015 for similar findings in another bird species). King
414 penguin chicks are raised in an unpredictable environment (high predation risk, socially
415 aggressive adults, inclement weather conditions), and are subject to periods of intermittent to
416 prolonged fasting early in life (Cherel & Le Maho, 1985). Thus, variation in parental care and
417 ability to efficiently provision and defend their offspring will have critical consequences on
418 offspring phenotype. Our results in king penguins suggests that selection on telomere length
419 might be sex-specific (see also Olsson et al. 2011b for similar finding in a lizard species).
420 However, why maternal and paternal contributions should differ is unclear. We do know that
421 feeding strategies differ between sexes in adult king penguins, but mostly during the winter
422 period (Saraux et al., 2012), which is outside of the experimental window of the present
423 study. However, subtle differences in incubation and feeding strategies between males and
424 females may lead mothers to display a larger effect on chick telomere length during
425 incubation and the first three months of growth. Whether this effect stems from the quality of
426 incubation and/or of the food provided specifically by the mother remains to be determined,
427 and focusing on food elements known to buffer deleterious effect on telomeres (*e.g.* dietary
428 antioxidants, Reichert & Stier, 2017) may provide new insights. In king penguin chicks,
429 telomeres seem to erode faster in rapidly growing individuals (Geiger et al., 2012). This
430 suggests that variation in maternal provisioning patterns early in life is likely another
431 important factor affecting chick telomere length. Adequate or more regular rates of food
432 provisioning by high quality adults may allow chicks to better balance out the allocation of
433 energy towards growth and other somatic compartments, without affecting body mass *per se*,
434 allowing higher telomere maintenance. Additionally, development does not only concern cell

multiplication and an increase in body mass but also physiological maturation. A recent study in birds suggested that maturation may be done at a cost of telomere loss (Criscuolo et al., 2019). Whether early maternal care may enable chicks to mature in a way that allows to better preserve telomere ends afterwards is intriguing and a call for further research. Finally, it is worth keeping in mind that, in this study, parental age was unknown. Because parental age can explain substantial variation in offspring telomere length (*e.g.* Criscuolo et al. 2017; Bauch et al. 2019), the reported association between foster and biological parental are probably an underestimation of any true association between parental and offspring telomere length.

Overall, our study provides experimental evidence that the quality of environmental rearing conditions mediated by the parents partly influence variation in offspring telomere length and survival in a long-lived seabird. Such an approach opens perspectives as to the finer characterization of the nature and timing of environmental effects conditioning individual survival chances in the wild.

ACKNOWLEDGMENTS

We are grateful to the field assistants who helped us with data collection in 2012-2013. This research was supported by the French Polar Research Institute (IPEV; program 119 ECONERGY). We are grateful to 5 anonymous reviewers for constructive and useful comments on previous drafts of the paper.

TABLES

Table 1. Standardized model estimates for the relationship between parental relative telomere length (RTL) and chick survival (binary 0 = failure / 1 = success) and phenotype (structural size, body condition and telomere length) early in the development (day 10 post-hatching). Significant effects have CI95 not overlapping 1 for the binomial model, and not overlapping 0 for linear models. All parents were included in the same model. Variance inflation factors (VIFs) are provided. The number of chicks (n) and dyads (N) are given. Sample sizes vary across models due to variation in chick mortality and/or difficulties at sampling blood from some chicks or amplifying DNA (telomeres) from some blood samples.

Chick survival and phenotype early during development (day 10)							
(A) Survival (binary 1/0)	*Odds ratio ± SE*	*CI*	*z*	*P*	*VIF*	R^2	*n (N)*
Intercept	19.99 ± 0.80	4.16 – 96.00	3.74	<0.001*			
***z* egg mass**	2.18 ± 0.58	0.70 – 6.78	1.35	0.178	1.30		
***z* RTL$_{biological♂}$**	0.15 ± 0.87	0.03 – 0.82	-2.18	0.029*	2.05	0.549	56 (28)
***z* RTL$_{biological♀}$**	1.68 ± 0.65	0.47 – 6.00	0.80	0.422	1.88		
***z* RTL$_{foster♂}$**	3.21 ± 0.73	0.77 – 13.37	1.61	0.108	1.96		
***z* RTL$_{foster♀}$**	3.25 ± 0.89	0.57 – 18.61	1.33	0.185	1.93		
(B) z Structural size	*Estimate ± SE*	*CI*	*t*	*P*	*VIF*	R^2	*n (N)*
Intercept	-0.17 ± 0.22	-0.39 – 0.17	-0.77	0.444			
***z* egg mass**	0.14 ± 0.23	-0.31 – 0.59	0.61	0.548	1.12		
***z* RTL$_{biological♂}$**	0.21 ± 0.29	-0.36 – 0.77	0.71	0.481	1.46	0.084	49 (28)
***z* RTL$_{biological♀}$**	-0.03 ± 0.26	-0.53 – 0.48	-0.10	0.921	1.33		
***z* RTL$_{foster♂}$**	0.32 ± 0.29	-0.25 – 0.88	1.10	0.271	1.32		
***z* RTL$_{foster♀}$**	0.02 ± 0.28	-0.53 – 0.56	0.06	0.956	1.40		
(C) z Body condition	*Estimate ± SE*	*CI*	*t*	*P*	*VIF*	R^2	*n (N)*
Intercept	0.06 ± 0.15	-0.25 – 0.36	0.39	0.696			
***z* egg mass**	0.13 ± 0.16	-0.18 – 0.44	0.83	0.409	1.12		
***z* RTL$_{biological♂}$**	0.30 ± 0.20	-0.09 – 0.68	1.51	0.139	1.47	0.078	49 (28)
***z* RTL$_{biological♀}$**	-0.20 ± 0.18	-0.54 – 0.15	-1.13	0.267	1.33		
***z* RTL$_{foster♂}$**	-0.03 ± 0.20	-0.41 – 0.36	-0.15	0.885	1.32		
***z* RTL$_{foster♀}$**	-0.20 ± 0.19	-0.57 – 0.17	-1.05	0.301	1.40		
(D) z RTL	*Estimate ± SE*	*CI*	*t*	*P*	*VIF*	R^2	*n (N)*
Intercept	-0.17 ± 0.13	-0.43 – 0.10	-1.24	0.222			
***z* egg mass**	-0.01 ± 0.15	-0.31 – 0.29	-0.04	0.968	1.21		
***z* RTL$_{biological♂}$**	0.14 ± 0.18	-0.20 – 0.49	0.81	0.423	1.54	0.335	40 (26)
***z* RTL$_{biological♀}$**	0.22 ± 0.17	-0.12 – 0.56	1.26	0.216	1.12		
***z* RTL$_{foster♂}$**	0.34 ± 0.18	-0.02 – 0.70	1.86	0.071	1.38		
***z* RTL$_{foster♀}$**	0.42 ± 0.17	0.08 – 0.76	2.42	0.021*	1.16		

Table 2. Standardized model estimates for the relationship between parental relative telomere length (RTL) and chick survival (binary 0 = failure / 1 = success) and phenotype (structural size, body condition and telomere length) late in the development (day 105 post-hatching; the end of the pre-winter growth phase). Significant effects have CI95 not overlapping 1 for the binomial model, and not overlapping 0 for linear models. All parents were included in the same model. Variance inflation factors (VIFs) are provided. The number of chicks (n) and dyads (N) are given. Sample sizes vary across models due to variation in chick mortality and/or difficulties at sampling blood from some chicks or amplifying DNA (telomeres) from some blood samples.

Chick survival and phenotype late in development (day 105)							
(A) Survival (binary 1/0)	*Odds ratio ± SE*	*CI*	*z*	*P*	*VIF*	R^2	*n (N)*
Intercept	3.35 ± 0.36	1.66 – 6.74	3.38	0.001*			
***z* egg mass**	1.38 ± 0.37	0.67 – 2.86	0.88	0.378	1.13		
***z* RTL$_{biological♂}$**	0.57 ± 0.45	0.24 – 1.37	-1.25	0.210	1.32	0.238	56 (28)
***z* RTL$_{biological♀}$**	1.00 ± 0.41	0.45 – 2.21	-0.00	0.999	1.25		
***z* RTL$_{foster♂}$**	2.99 ± 0.44	1.26 – 7.08	2.49	0.013*	1.30		
***z* RTL$_{foster♀}$**	1.15 ± 0.42	0.50 – 2.64	0.33	0.745	1.29		
(B) z Structural size	*Estimate ± SE*	*CI*	*t*	*P*	*VIF*	R^2	*n (N)*
Intercept	0.01 ± 0.17	-0.32 – 0.34	0.07	0.944			
***z* egg mass**	0.00 ± 0.16	-0.30 – 0.31	0.08	0.978	1.05		
***z* RTL$_{biological♂}$**	0.15 ± 0.18	-0.20 – 0.50	0.91	0.412	1.30	0.050	41 (27)
***z* RTL$_{biological♀}$**	0.10 ± 0.19	-0.27 – 0.47	0.60	0.592	1.32		
***z* RTL$_{foster♂}$**	-0.15 ± 0.20	-0.54– 0.25	-0.63	0.474	1.19		
***z* RTL$_{foster♀}$**	-0.21 ± 0.20	-0.60 – 0.19	-1.09	0.308	1.38		
(C) z Body condition	*Estimate ± SE*	*CI*	*t*	*P*	*VIF*	R^2	*n (N)*
Intercept	-0.04 ± 0.19	-0.42 – 0.34	-0.22	0.825			
***z* egg mass**	0.08 ± 0.18	-0.27 – 0.42	0.44	0.665	1.05		
***z* RTL$_{biological♂}$**	0.06 ± 0.20	-0.32 – 0.45	0.31	0.757	1.26	0.078	41 (27)
***z* RTL$_{biological♀}$**	-0.08 ± 0.21	-0.50 – 0.34	-0.37	0.716	1.46		
***z* RTL$_{foster♂}$**	0.25 ± 0.22	-0.19 – 0.68	1.11	0.274	1.15		
***z* RTL$_{foster♀}$**	-0.32 ± 0.23	-0.76 – 0.13	-1.39	0.174	1.53		
(D) z RTL	*Estimate ± SE*	*CI*	*t*	*P*		R^2	*n (N)*
Intercept	-0.16 ± 0.15	-0.45 – 0.13	-1.09	0.288			
***z* egg mass**	0.01 ± 0.16	-0.31 – 0.33	0.09	0.932	1.19		
***z* RTL$_{biological♂}$**	0.08 ± 0.18	-0.28 – 0.43	0.44	0.664	1.40	0.330	40 (26)
***z* RTL$_{biological♀}$**	0.31 ± 0.19	-0.05 – 0.67	1.67	0.104	1.23		
***z* RTL$_{foster♂}$**	0.28 ± 0.19	-0.09 – 0.65	1.48	0.148	1.25		
***z* RTL$_{foster♀}$**	0.54 ± 0.19	0.18 – 0.91	2.90	0.007*	1.28		

480 **Table 3.** Standardized linear mixed model estimates for the relationship between parental
481 relative telomere lengths (RTL) and chick change in relative telomere length over growth (i.e.
482 between days 10 and 105 post-hatching). All parents were included in the same model.
483 Variance inflation factors (VIFs) are provided. The number of chicks (n) and dyads (N) are
484 given.

485

Chick RTL change over growth ($RTL_{chick105} - RTL_{chick10}$)							
z RTL change	*Estimate ± SE*	*CI*	*t*	*P*	*VIF*	R^2	*n (N)*
Intercept	0.02 ± 0.17	-0.31 – 0.34	0.10	0.924			
***z* RTL$_{biological♂}$**	-0.11 ± 0.21	-0.53 – 0.30	-0.53	0.597	1.41		
***z* RTL$_{biological♀}$**	0.04 ± 0.21	-0.38 – 0.46	0.20	0.844	1.09	0.019	40 (26)
***z* RTL$_{foster♂}$**	-0.08 ± 0.23	-0.54 – 0.37	-0.37	0.717	1.38		
***z* RTL$_{foster♀}$**	0.05 ± 0.22	-0.38 – 0.47	0.21	0.831	1.14		

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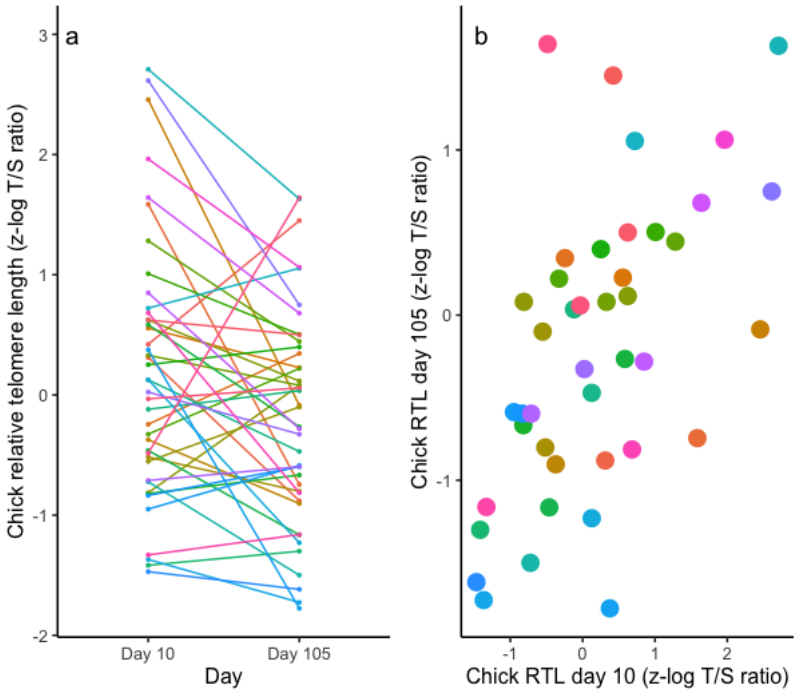

Fig. 1. King penguin chick relative telomere length (RTL, T/S ratio) dynamics in early life. RTL was log transformed, and all values were standardized (z-scores). (a) Individual trajectories in RTL between days 10 and 105, i.e. the pre-winter growth period. (b) Relationship between RTL values at day 10 and 105. Different colours indicate different birds.

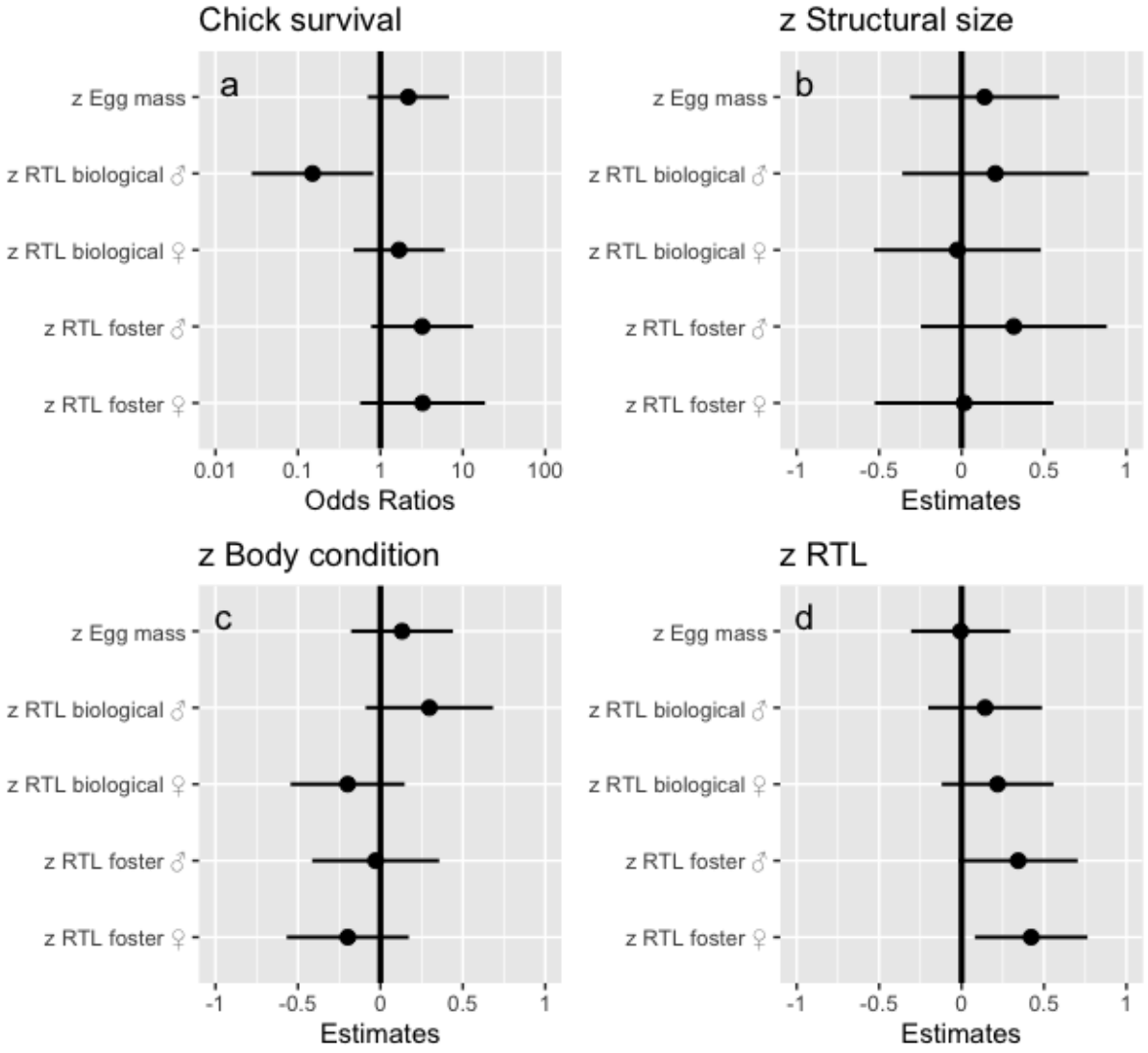

Fig. 2. Relationships between king penguin parental telomere length (RTL) and chick survival and phenotype early in development (day 10 post-hatching). All parents were included in the same model, and different mixed models were run for (a) chick survival (binary 0/1); (b) chick structural size (principal components axis, see Methods); (c) chick body condition (see Methods); and (d) chick RTL. Standardized mixed model estimates are given with 95% CI. Significant effects have CI_{95} not overlapping 1 for the binomial model, and not overlapping 0 for linear models. Positive and negative effects fall to the right and left of the vertical line, respectively. RTL is expressed as log (T/S ratio), and all variables were standardized (*z*-scores) priori to analyses.

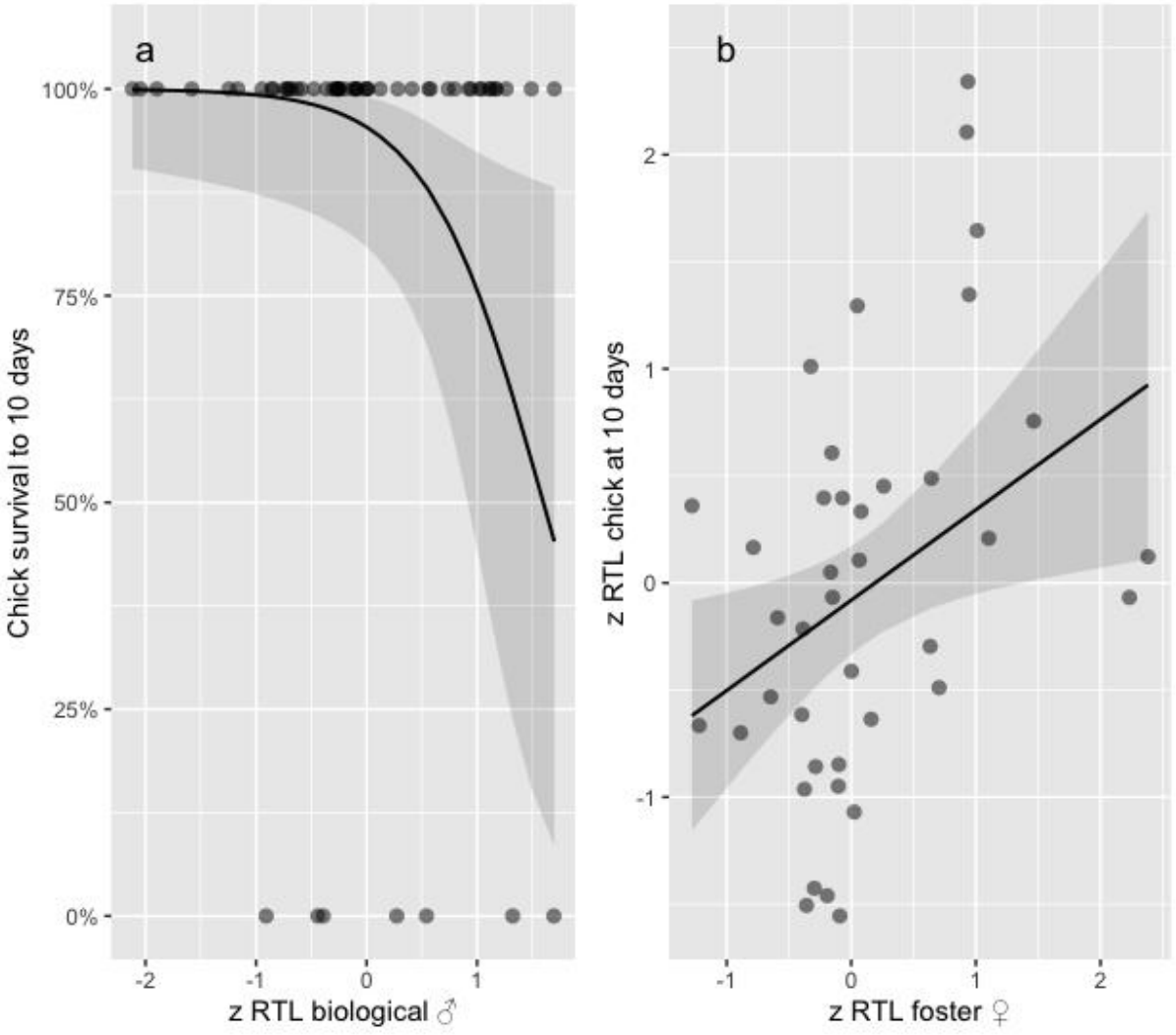

Fig. 3. (a) Predicted probability and 95% CI of chick survival at 10 days as a function of biological male relative telomere length (RTL). (b) Relationship between foster female RTL and chick RTL at 10 days. RTL is expressed as log (T/S ratio), and was standardized (*z*-scores) priori to analyses.

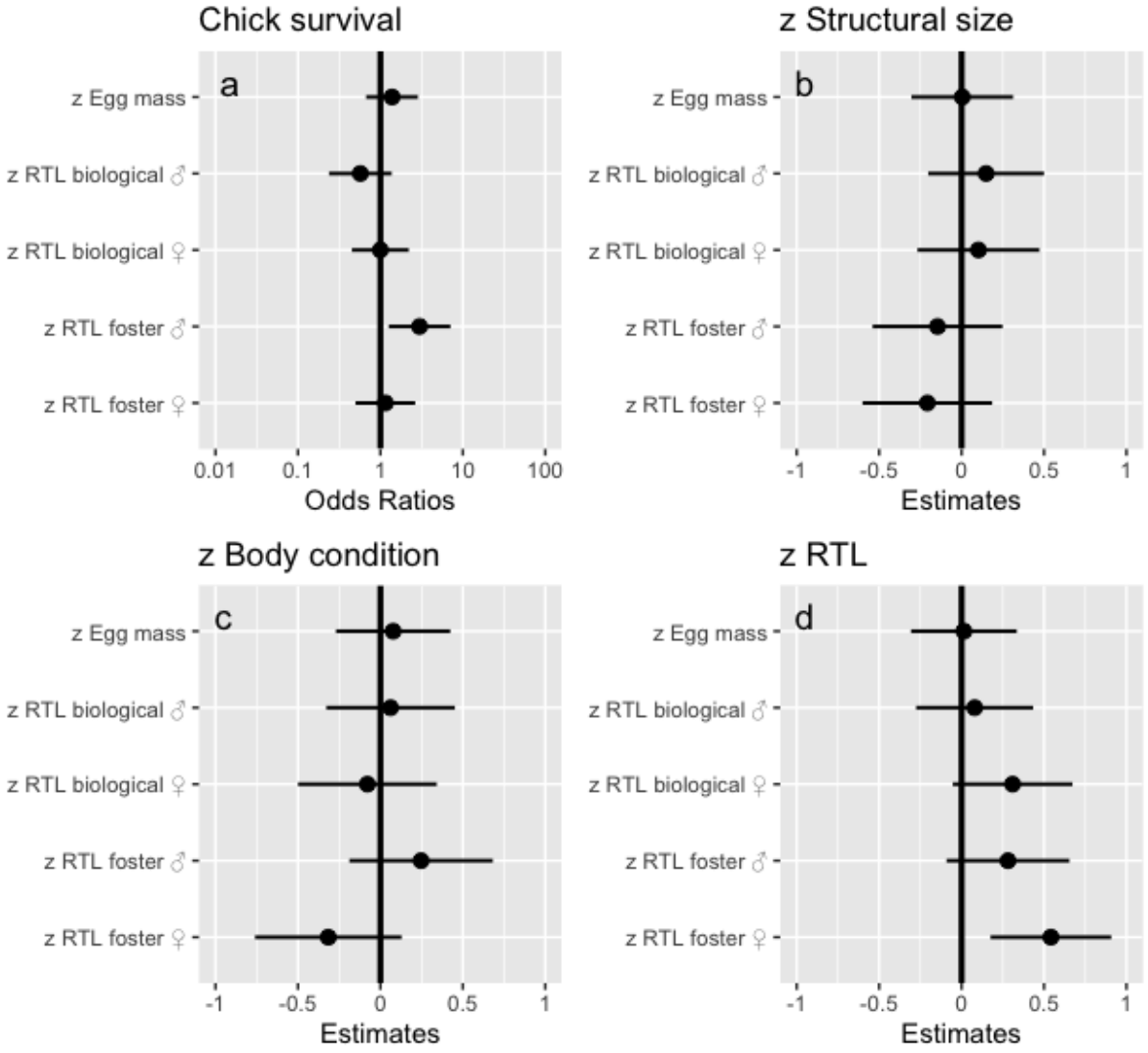

Fig. 4. Relationships between king penguin parental telomere length (RTL) and chick survival and phenotype late in the development (day 105 post-hatching, the end of the pre-winter growth phase). All parents were included in the same model, and different mixed models were run (a) chick survival (binary 0/1); (b) chick structural size (principal components axis, see Methods); (c) chick body condition (see Methods); and (d) chick RTL. Standardized mixed model estimates are given with 95% CI. Significant effects have CI_{95} not overlapping 1 for the binomial model, and not overlapping 0 for linear models. Positive and negative effects fall to the right and left of the vertical line, respectively. RTL is expressed as log (T/S ratio), and all variables were standardized (*z*-scores) priori to analyses.

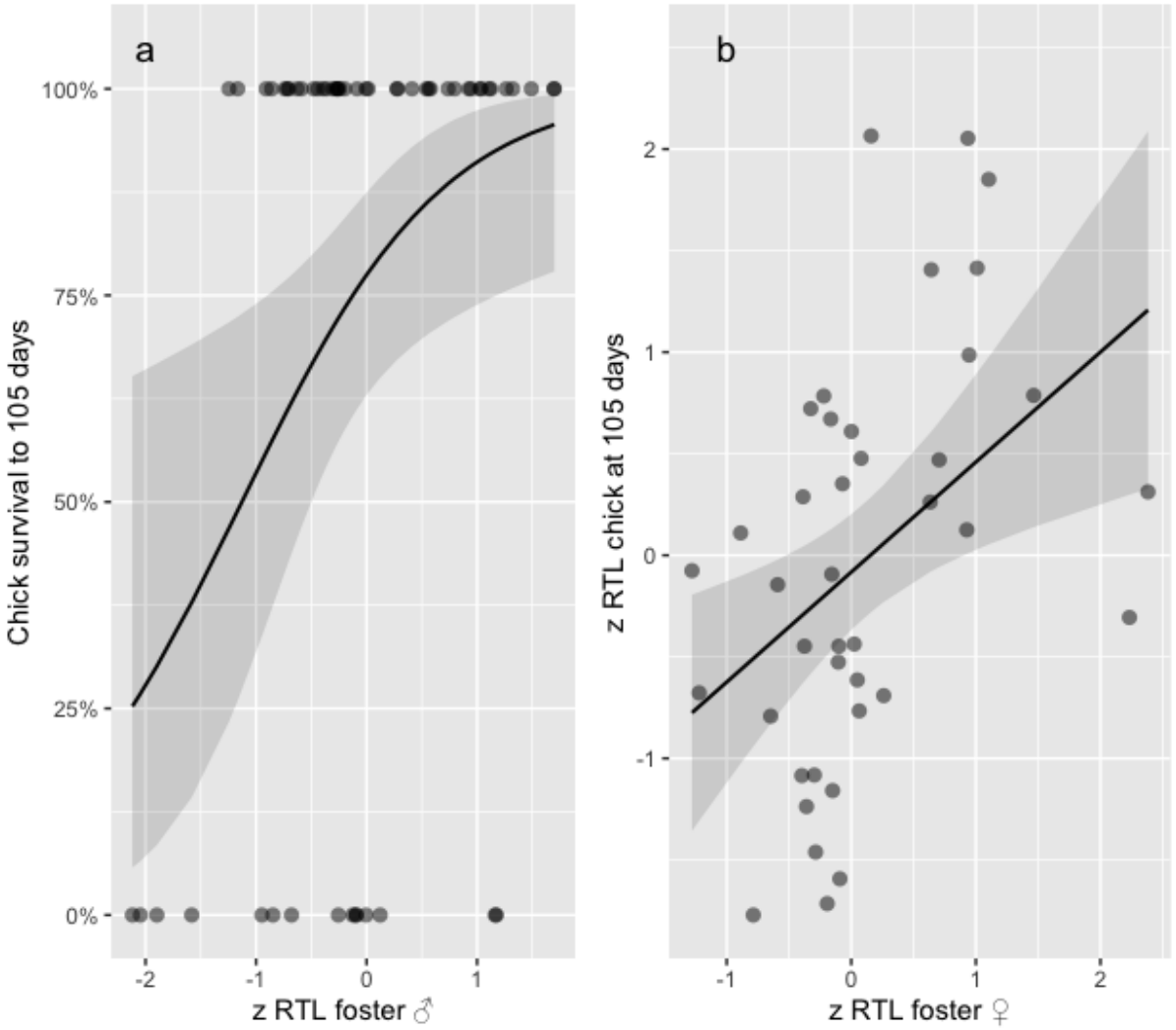

Fig. 5. (a) Predicted effect and 95% CI of chick survival at 105 days as a function of foster male relative telomere length (RTL). (b) Relationship between foster female RTL and chick RTL at 105 days. RTL is expressed as log (T/S ratio), and was standardized (*z*-scores) priori to analyses.

CONFLICT OF INTEREST

None declared

AUTHOR CONTRIBUTION

J.-P.R. is the PI of the polar research program 119. V.A.V. and P.B. conceived the experiment; Q.S., A.S., L.D, E.L. conducted the experiment, Q.S., S.Z. and F.C. extracted the DNA and performed the qPCR measurements and RTL analyses, F.C. and V.A.V. ran the statistical analyses and wrote a first version of the manuscript. All authors drafted the final manuscript and gave their approval for publication.

DATA ACCESSIBILITY

The data associated with this manuscript are available online at figshare xxxxxxx.

ORCID

Vincent A. Viblanc http://orcid.org/0000-0002-4953-659X

Quentin Schull http://orcid.org/0000-0001-9297-3376

Antoine Stier https://orcid.org/0000-0002-5445-5524

Jean-Patrice Robin http://orcid.org/0000-0002-9500-2724

Sandrine Zahn http://orcid.org/0000-0001-9303-4223

François Criscuolo http://orcid.org/0000-0001-8997-8184

Pierre Bize http://orcid.org/0000-0002-6759-4371

REFERENCES

1. Angelier, F., Weimerskirch, H., Barbraud, C., & Chastel, O. (2019). Is telomere length a molecular marker of individual quality? Insights from a long-lived bird. *Functional Ecology*, *33*, 1076-1087.
2. Asghar, M., Bensch, S., Tarka, M., Hansson, B., & Hasselquist, D. (2014). Maternal and genetic factors determine early life telomere length. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20142263-20142263.
3. Atema, E., Mulder, E., Dugdale, H. L., Briga, M., Van Noordwijk, A. J., & Verhulst, S. (2015). Heritability of telomere length in the Zebra Finch. *Journal of Ornithology*, *1*, 11.
4. Bateson, M., Eisenberg, D. T., & Nettle, D. (2019). Controlling for baseline telomere length biases estimates of the rate of the rate of telomere attrition. *Royal Society Open Science*, *6*, 190937.
5. Bauch, C., Becker, P. H., & Verhulst, S. (2013). Telomere length reflects phenotypic quality and costs of reproduction in a long-lived seabird. *Proceedings of the Royal Society B: Biological Sciences*, *280*, 20122540-20122540.
6. Bauch, C., Boonekamp, J. J., Korsten, P., Mulder, E., & Verhulst, S. (2019). Epigenetic inheritance of telomere length in wild birds. *PLoS Genetics*, *15*, e1007827.
7. Bauch, C., Riechert, J., Verhulst, S., & Becker, P. H. (2016). Telomere length reflects reproductive effort indicated by corticosterone levels in a long-lived seabird. *Molecular Ecology*, *25*, 5785-5794.
8. Becker, P. J. J., Reichert, S., Zahn, S., Hegelbach, J., Massemin, S., Keller, L. F., Postma, E. & Criscuolo, F. (2015). Mother-offspring and nest-mate resemblance but no heritability in early-life telomere length in white-throated dippers. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20142924-20142924.
9. Belmaker, A., Hallinger, K. K., Glynn, R. A., Winkler, D. W., & Haussmann, M. F. (2019). The environmental and genetic determinants of chick telomere length in Tree Swallows (*Tachycineta bicolor*). *Ecology and Evolution*, *9*, 8175-8186.
10. Bize, P., Roulin, A., & Richner, H. (2002). Covariation between egg size and rearing condition determines offspring quality: an experiment with the Alpine swift. *Oecologia*, 132, 231-234.
11. Bize, P., Criscuolo, F., Metcalfe, N. B., Nasir, L., & Monaghan, P. (2009). Telomere dynamics rather than age predict life expectancy in the wild. *Proceedings of the Royal Society B: Biological Sciences*, *276*, 1679-1683.
12. Boonekamp, J. J., Mulder, G. A., Salomons, H. M., Dijkstra, C. & Verhulst, S. (2014). Nestling telomere shortening, but not telomere length, reflects developmental stress and predicts survival in wild birds. *Proceedings of the Royal Society of London B*, 281, 20133287.
13. Cawthon, R. M. (2002). Telomere measurement by quantitative PCR. *Nucleic Acids Research*, 30, e47.
14. Cerchiara, J. A., Risques, R. A., Prunkard, D., Smith, J. R., Kane, O. J., & Boersma, P. D. (2017). Magellanic penguin telomeres do not shorten with age with increased reproductive effort, investment, and basal corticosterone. *Ecology and Evolution*, *7*, 5682-5691.
15. Chatelin, M., Drobniak, S. M. & Szulkin, M. (2019) The association between stressors and telomeres in non-human vertebrates: a meta-analysis. *Ecology Letters*, 23, 381-398.

16. Cherel, Y. & Le Maho, Y. (1985). Five months of fasting in king penguin chicks: body mass loss and fuel metabolism. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 249, R387-R392.
17. Criscuolo, F., Cornell, A., Zahn, S., & Williams, T. D. (2019). Oxidative status and telomere length are related to somatic and physiological maturation in chicks of European starlings (*Sturnus vulgaris*). *The Journal of Experimental Biology, 222*, jeb204719.
18. Criscuolo, F., Zahn, S., & Bize, P. (2017). Offspring telomere length in the long lived Alpine swift is negatively related to the age of their biological father and foster mother. *Biology Letters, 13*, 20170188.
19. Delignette-Muller, M. L., & Dutang, C. (2015). fitdistrplus: An R package for fitting distributions. *Journal of Statistical Software*, 64, 1-34.
20. Descamps, S., Gauthier-Clerc, M., Le Bohec, C., Gendner, J.-P. & Le Maho, Y. (2005). Impact of predation on king penguin *Aptenodytes patagonicus* in Crozet Archipelago. *Polar Biology, 28*, 303-310.
21. Dugdale, H. L., & Richardson, D. S. (2018). Heritability of telomere variation: it is all about the environment! *Philosophical Transactions of the Royal Society B: Biological Sciences, 373*, 20160450.
22. Eastwood, J. R., Hall, M. L., Teunissen, N., Kingma, S. A., Aranzamendi, N. H., Fan, M., Roast, M., Verhulst, S. & Peters, A. (2019). Early-life telomere length predicts lifespan and lifetime reproductive success in a wild bird. *Molecular Ecology*, 28, 1127-1137.
23. Eisenberg, D. T. A. (2019). Paternal age at conception effects on offspring telomere length across species—What explains the variability? *PLoS Genetics, 15*, e1007946.
24. Eisenberg, D. T. A., Kuzawa, C. W., & Hayes, M. G. (2015). Improving qPCR telomere length assays: Controlling for well position effects increases statistical power. *American Journal of Human Biology, 27*, 570-575.
25. Fairlie, J., Holland, R., Pikington, J. G., Pemberton, J. M., Harrington, L., & Nussey, D. H. (2016). Lifelong leukocyte telomere dynamics and survival in a free-living mammal. *Aging Cell, 15*, 140-148.
26. Forslund, P. & Pärt, T. (1995). Age and reproduction in birds: Hypotheses and tests. *Trends in Ecology & Evolution*, 10, 374-378.
27. Geiger, S., Le Vaillant, M., Lebard, T., Reichert, S., Stier, A., Le Maho, Y., & Criscuolo, F. (2012). Catching-up but telomere loss: half-opening the black box of growth and ageing trade-off in wild king penguin chicks. *Molecular Ecology, 21*(6), 1500-1510.
28. Gomes, N. M. V., Shay, J. W., & Wright, W. E. (2010). Telomere biology in Metazoa. *FEBS Letters, 584*, 3741-3751.
29. Graakjaer, J., Pascoe, L., der-Sarkissian, H., Thomas, G., Kolvraa, S., Christensen, K., & Londono-Valleja, J. A. (2004). The relative lengths of individual telomeres are defined in the zygote and strictly maintained during life. *Aging Cell, 3*, 97-102.
30. Hanssen , L. M., Schutte, N. S., Malouff, J. M., & Epel, E. S. (2017). The relationship between childhood psychosocial stressor level and telomere length: a meta-analyses. *Health Psychology Research*, 5, 6378.
31. Haussmann, M. F., Longenecker, A. S., Marchetto, N. M., Juliano, S. A., & Bowden, R. M. (2012). Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. *Proceedings of the Royal Society B: Biological Sciences*, 279, 1447-1456.

32. Heidinger, B. J., Blount, J. D., Boner, W., Griffiths, K., Metcalfe, N. B., & Monaghan, P. (2012). Telomere length in early life predicts lifespan. *Proceedings of the National Academy of Sciences, 109*, 1742-1748.
33. Jouventin, P., & Dobson, F. S. (2017). Why penguins communicate: the evolution of visual and vocal signals. *Academic Press*.
34. Krist, M. (2011). Egg size and offspring quality: a meta-analysis in birds. *Biological Reviews*, 86, 692-716
35. Lecomte, V. J., Sorci, G., Cornet, S., Jaeger, A., Faivre, B., Arnoux, E., Gaillard, M., Trouvé, C., Besson, D., Chastel, O., & Weimerskirch, H. (2010). Patterns of aging in the long-lived wandering albatross. *Proceedings of the National Academy of Science of the USA*, 107, 6370-6375.
36. Le Vaillant, M., Viblanc, V. A., Saraux, C., Le Bohec, C., Le Maho, Y., Kato, A., Criscuolo, F. & Ropert-Coudert, Y. (2015). Telomere length reflects individual quality in free-living adult king penguins. *Polar Biology*, 38, 2059-2067.
37. Levy, M. Z., Allsopp, R. C., Futcher, A. B., Greider, C. W., & Harley, C. B. (1992). Telomere end-replication problem and cell aging. *Journal of Molecular Biology, 225*, 951-960.
38. McCarty, R. (2017). Cross-fostering: Elucidating the effects of gene×environment interactions on phenotypic development. *Neuroscience & Biobehavioral Reviews, 73*, 219-254.
39. Metcalfe, N., & Monaghan, P. (2003). Growth versus lifespan: perspectives from evolutionary ecology. *Experimental Gerontology, 38*, 935-940.
40. Monaghan, P., & Ozanne, S. E. (2018). Somatic growth and telomere dynamics in vertebrates: relationships, mechanisms and consequences. *Philosophical Transactions of the Royal Society B: Biological Sciences, 373*, 20160446.
41. Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews, 85*, 935-956.
42. Nettle, D., Monaghan, P., Gillespie, R., Brilot, B., Bedford, T., & Bateson, M. (2015). An experimental demonstration that early-life competitive disadvantage accelerates telomere loss. *Proceedings of the Royal Society B: Biological Sciences, 282*, 20141610-20141610.
43. Noguera, J.C. & Velando, A. (2019) Reduced telomere length in embryos exposed to predator cues, *The Journal of Experimental Biology*, 222, jeb216176.
44. Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T., & Blomqvist, D. (2011a). Sex differences in sand lizard telomere inheritance: paternal epigenetic effects increases telomere heritability and offspring survival. *Plos One, 6*, e17473.
45. Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T., Miller, E., & Blomqvist, D. (2011b). Sexual differences in telomere selection in the wild. *Molecular Ecology*, 20, 2085-2099.
46. Olsson, O. (1998). Divorce in King Penguins: Asynchrony, expensive fat storing and ideal free mate choice. *Oikos, 83*, 574-581.
47. Reichert, S., Rojas, E. R., Zahn, S., Robin, J. P., Criscuolo, F., & Massemin, S. (2015). Maternal telomere length inheritance in the king penguin. *Heredity, 114*, 10-16.
48. Reichert, S., & Stier, A. (2017). Does oxidative stress shorten telomeres in vivo? A review. *Biology Letters, 13*, 20170463.
49. Salomons, H. M., Mulder, G. A., van de Zande, L., Haussmann, M. F., Linskens, M. H. K., & Verhulst, S. (2009). Telomere shortening and survival in free-living corvids. *Proceedings of the Royal Society B: Biological Sciences, 276*, 3157-3165.

50. Saraux, C., Friess, B., Le Maho, Y., & Le Bohec, C. (2012). Chick-provisioning strategies used by king penguins to adapt to a multiseasonal breeding cycle. *Animal Behaviour, 84*, 675-683.
51. Saulnier, A., Bleu, J., Boos, A., El Masoudi, I., Ronot, P., Zahn, S., Del Nero, M. & Massemin, S. (2020). Consequences of trace metal cocktail exposure in zebra finch (*Taenopygia guttata*) and the effect of calcium supplementation. *Ecotoxicology and Environmental Safety*, 193, 110357.
52. Schull, Q., Viblanc, V. A., Dobson, F. S., Robin, J. P., Zahn, S., Cristofari, R., Bize, P., & Criscuolo, F. (2018). Assortative pairing by telomere length in king penguins (*Aptenodytes patagonicus*) and relationships with breeding success. *Canadian Journal of Zoology, 96*, 639-647.
53. Schulte-Hostedde, A. I., Zinner, B., Millar, J. S., & Hickling, G. J. (2005). Restitution of mass–size residuals: validating body condition indices. *Ecology, 86*, 155-163.
54. Soler, J. J., Ruiz-Castellano, C., Figuerola, J., Martín-Vivaldi, M., Martínez-de la Puente, J., Ruiz-Rodríguez, M., & Tomás, G. (2017). Telomere length and dynamics of spotless starling nestlings depend on nest-building materials used by parents. *Animal Behaviour, 126*, 89-100.
55. Stier, A., Viblanc, V. A., Massemin-Challet, S., Handrich, Y., Zahn, S., Rojas, E. R., Saraux, C., Le Vaillant, M., Prud'homme, O., Grosbellet, E., Robin, J.-P., Bize, P., & Criscuolo, F. (2014). Starting with a handicap: phenotypic differences between early- and late-born king penguin chicks and their survival correlates. *Functional Ecology, 28*, 601-611.
56. Stier, A., Reichert, S., Criscuolo, F. & Bize, P. (2015). Red blood cells open promising avenues for longitudinal studies of ageing in laboratory, non-model and wild animals. *Experimental Gerontology*, *71*, 118-134.
57. Stier, A., Metcalfe, N. B. & Monaghan, P. (2019). Ageing before birth: pace and stability of prenatal growth affect telomere dynamics. *bioRxiv*, 809087.
58. Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution, 8*, 1639-1644.
59. Stonehouse, B. (1960). The king penguin Aptenodytes patagonica of South Georgia. I. Breeding behaviour and development. *Scientific Report of the Falkland Islands Dependencies Survey*, *23*, 1-81.
60. Tarry-Adkins, J. L., Chen, J. H., Smith, N. S., Jones, R. H., Cherif, H., & Ozanne, S. E. (2009). Poor maternal nutrition followed by accelerated postnatal growth leads to telomere shortening and increased markers of cell senescence in rat islets. *The FASEB Journal, 23*, 1521-1528.
61. Tjur, T. (2009). Coefficients of determination in logistic regression models - A new proposal: the coefficient of discrimination. *The American Statistician*, 63, 366-372.
62. Tomiyama, A. J., O'Donovan, A., Lin, J., Puterman, E., Lazaro, A., Chan, J., Dhabhar, F. S., Wolkowitz, O., Kirschbaum, C., Blackburn E. & Epel, E. (2012). Does cellular aging relate to patterns of allostasis? An examination of basal and stress reactive HPA axis activity and telomere length. *Physiology & Behavior*, 106, 40-45.
63. Tricola, G. M., Simons, M. J. P., Atema, E., Boughton, R. K., Brown, J. L., Dearborn, D. C., Dikovy, G., Eimes, J. A., Huntington, C. E., Kitaysky, A. S., Juola, F. A., Lank, D. B., Litwa, H. P., Mulder, E. G. A, Nisbet, I. C. T., Okanoya, K., Safran, R. J., Schoech, S. J., Schreiber, E. A., Thompson, P. M., Verhulst, S., Wheelwright, N. T., Winkler, D. W., Young, R., Vleck, C. M., & Haussmann, M. F. (2018). The rate of telomere loss is related to maximum lifespan in birds. *Philosophical Transactions of the Royal Society B: Biological Sciences, 373*, 20160445.

64. Verhulst, S., Dalgård, C., Labat, C., Kark, J. D., Kimura, M., Christensen, K., Toupance, S., Aviv, A., Kyvik, K. O., & Benetos, A. (2016). A short leucocyte telomere length is associated with development of insulin resistance. *Diabetologia*, *59*, 1258-1265.
65. Weimerskirch, H., Stahl, J. C., & Jouventin, P. (1992). The breeding biology and population dynamics of King Penguins *Aptenodytes patagonica* on the Crozet Islands. *Ibis*, *134*, 107-117.
66. Whittemore, K., Vera, E., Martínez-Nevado, E., Sanpera, C., & Blasco, M. A. (2019). Telomere shortening rate predicts species life span. *Proceedings of the National Academy of Sciences*, 201902452.
67. Wilson, A. J., & Nussey, D. H. (2010). What is individual quality? An evolutionary perspective. *Trends in Ecology & Evolution*, *25*, 207-214.
68. Young, R. C., Kitaysky, A. S., Barger, C. P., Dorresteijn, I., Ito, M., & Watanuki, Y. (2015). Telomere length is a strong predictor of foraging behavior in a long-lived seabird. *Ecosphere*, *6*, art39.
69. Young, R. C., Barger, C. P., Dorresteijn, I., Haussmann, M. F., & Kitaysky, A. S. (2016). Telomere length and environmental conditions predict stress levels but not parental investment in a long-lived seabird. *Marine Ecology Progress Series*, *556*, 251-259.
70. Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, *1*, 3-14.