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Abstract

1. The age of the parents at the time of offspring production can influence offspring longevity, but the underlying mechanisms remain poorly understood. The effect of parental age on offspring telomere dynamics (length and loss rate) is one mechanism that could be important in this context.
2. Parental age might influence the telomere length that offspring inherit or age-related differences in the quality of parental care could influence the rate of offspring telomere loss. However, these routes have generally not been disentangled.
3. Here we investigated whether parental age was related to offspring telomere dynamics using parents ranging in age from 2 to 22 years old in a free-living population of a long-lived seabird the European shag (*Phalacrocorax aristotelis*). By measuring the telomere length of offspring at hatching and towards the end of the post-natal growth period, we could assess whether any potential parental age effect was confined to the post-natal rearing period.
4. There was no effect of maternal or paternal age on the initial telomere length of their chicks. However, chicks produced by older mothers and fathers experienced significantly greater telomere loss during the post-natal nestling growth period. We had relatively few nests in which the ages of both parents were known, and individuals in this population mate assortatively with respect to age. Thus, we could not conclusively determine whether the parental age effect was due to maternal age, paternal age, or both; however, it appears that the effect is stronger in mothers.
5. These results demonstrate that in this species, there was no evidence that parental age was related to offspring hatching telomere length. However, telomere loss during nestling growth was reduced in the offspring of older parents. This could be due to an age-related deterioration in the quality of the environment that parents provide, or because parents that invest less in offspring rearing live to an older age.

Introduction

Parental age at the time of offspring production can influence offspring lifespan in diverse taxa (Lansing 1947; Priest, Mackowiak & Promislow 2002; Fox, Bush & Wallin 2003; Tarin *et al.* 2005; Bouwhuis *et al.* 2010; Ducatez *et al.* 2012; Gillespie, Russell & Lummaa 2013). However, the mechanisms underlying this effect remain poorly understood. Telomere dynamics, both length and loss rate, might be of critical importance in this context. Telomeres are highly conserved, tandem repeats of a short, non-coding DNA sequence (TTAGGG in all vertebrates) at the ends of eukaryotic chromosomes. Together with *shelterin* proteins, telomeres mark the chromosome ends and form protective caps that shield the coding sequences from loss during normal cell division (Blackburn 2005). Telomeres can limit cellular lifespan because, once telomeres become critically short, cells enter a state of replicative senescence and either die or remain in tissues and secrete inflammatory compounds that impair tissue function (Blackburn 2005). Both of these outcomes are thought to contribute to age-related declines in tissue function and organismal senescence (Aubert & Lansdorp 2008). In support of this, in many organisms, telomere length has been shown to decrease with age in diverse tissues (Monaghan & Haussmann 2006; Monaghan 2010). Further, in a number of species, telomere length and loss rate are related to longevity; individuals with longer telomeres and/or slower rates of telomere loss have longer lives (Cawthon *et al.* 2003; Monaghan & Haussmann 2006; Bize *et al.* 2009; Monaghan 2010; Heidinger *et al.* 2012; Barrett *et al.* 2013; Boonekamp *et al.* 2014; Asghar 2015).

Some of the variation in telomere dynamics is genetically inherited (Broer *et al.* 2013), but environmental factors during development (Monaghan & Haussmann 2006; Monaghan 2010; Entringer *et al.* 2011; Boonekamp *et al.* 2014; Herborn *et al.* 2014), including the age of the parent, are also likely to be important (Unryn, Cook & Riabowol 2005; De Meyer *et al.* 2007; Njajou *et al.* 2007; Olsson *et al.* 2011; Eisenberg, Hayes & Kuzawa 2012; Broer *et al.* 2013; Asghar 2015). Parental age could influence offspring telomere length both pre- and post-fertilisation (Haussmann & Heidinger 2015). For example, older parents might produce gametes with shorter telomeres than younger parents due to variation in the cell division history, exposure of gametes to damaging environmental effects, or in the fertilising power of sperm with different telomere lengths (Keefe, Liu & Marquard 2007).

The quality of the embryonic environment has also been shown to influence offspring telomere length at birth (Entringer *et al.* 2012; Haussmann *et al.* 2012; Broer *et al.* 2013) and might change with parental age. In birds, the limited published research to date suggests that there

is a parental age effect (Voillemot *et al.* 2012) and that maternal, rather than paternal, age may be more important (Horn *et al.* 2011; Reichert *et al.* 2014; Asghar *et al.* 2015). In great reed warblers, maternal age influenced offspring post-natal telomere length, but this effect depended on the infection status of the mother (Asghar 2015). As they grew older, mothers uninfected by malarial parasites produced offspring with longer telomeres, whereas as infected mothers grew older, they produced offspring with shorter telomeres. In other vertebrates, there is evidence that paternal effects may be more important. In sand lizards, there is evidence of an effect of paternal age on offspring telomere length; sons produced by older fathers had shorter telomeres in adulthood (Olsson *et al.* 2011). In humans, both the average telomere length in sperm and in offspring, have been shown to increase with paternal age (De Meyer *et al.* 2007; Kimura *et al.* 2008; Eisenberg, Hayes & Kuzawa 2012; Prescott *et al.* 2012). Importantly however, the offspring in all of these studies were sampled once, either near the end of the post-natal growth period or in adulthood, thus it is not possible to determine whether the observed relationships are primarily due to pre-natal effects of parental age, post-natal effects of parental age or both.

If it is the quality of the post-natal environment that matters, the effect of parental age on offspring telomere length might depend on how the quality of the rearing environment varies with parental age. In many organisms, parental age influences the habitat quality and/or the care provided for offspring (Clutton-Brock 1991; Forslund & Part 1995; Froy *et al.* 2013; Nussey *et al.* 2013; Hayward *et al.* 2014; Oro *et al.* 2014; Rabon 2014). Recent research in birds suggests that exposure to stressors, such as increased competition for limited parental resources or increased disturbance, accelerates telomere loss in nestlings (Nettle *et al.* 2013; Boonekamp *et al.* 2014; Herborn *et al.* 2014). Therefore, if the quality of resources or care for offspring changes with parental age, this might influence the rate of offspring telomere attrition.

Here we investigated the influence of parental age on offspring telomere dynamics in a free-living population of a long-lived seabird, the European shag (*Phalacrocorax aristotelis*). We sampled offspring produced by known-aged adults (ranging in age from 2-22 years old) at hatching and again close to fledging, enabling us to examine whether any parental age effects were confined to the offspring rearing period, or whether they were already present when the offspring hatched.

Materials and methods

(a) Study subjects

Research was conducted between May and July 2010 and 2011, on a free-living population of European shags (*Phalacrocorax aristotelis*) that breed on the Isle of May National Nature Reserve in the Firth of Forth, Scotland (56° 11′ 9″ N, 2° 33′ 27″ W). This population of shags has been studied for over 30 years and the chicks are ringed annually. Two-year old birds can also be accurately aged based on plumage characteristics (Potts 1971). Consequently, the ages of many of the adults that breed on the island are known. European shags are relatively long-lived (the oldest bird recorded in this population was 22 years old), socially monogamous seabirds that provide extensive care for their offspring (Wanless 1997). Here we focused on nests in which the age of least one of the parents was known. The ages of the parents are highly correlated in this species (see below) and thus we could not conclusively separate maternal and paternal age effects. Female shags lay clutches that range from 1-5 eggs; the modal clutch size for this population is 3 eggs (Daunt *et al.* 1999). Both parents incubate the eggs and the altricial chicks hatch asynchronously after approximately 35 days (Potts, Coulson & Deans 1980). Both parents then brood and feed the chicks and the chicks leave the nest approximately 50 days post-hatching (Daunt *et al.* 1999).

(b) Sampling

To measure offspring telomere length, small blood samples were collected from the chicks' tarsal veins with a needle and syringe within 2 days of hatching (n = 311 chicks from n = 134 nests where the age of at least one parent was known). Chicks were individually marked with coloured tape until they were large enough to be given permanent metal rings. To measure growth, chicks were weighed at hatching, and at the start and peak of the linear growth phase, approximately at days 10 and 30 respectively (Daunt *et al.* 2001). To measure telomere loss, a second blood sample was collected at the last growth measurement (between age 25-35 days). Telomere length based on the first blood sample is referred to as 'hatching telomere length', and the actual ages at which both blood samples were collected are included in the analyses. Blood samples were stored on ice in the field for less than 6 hours, centrifuged at 2000 rpm for 10 minutes, separated into plasma and red blood cell fractions, and stored at -20°C for less than two months before being transferred on dry ice to a -80 °C freezer.

(c) Telomere measurements

Telomere length was measured in red blood cells (RBC). RBCs are nucleated in birds, and represent a highly proliferative tissue that is well suited to telomere analysis (Nussey *et al.* 2014). The DNA was extracted from 4 µl RBC samples in 196 µl of phosphate buffered saline solution using Macherey-Nagle Whole Blood Kits (Macherey-Nagel, Bethlehem, PA, USA) and following the manufacturer's instructions. We measured the quantity of the extracted DNA with a nanodrop 8000 spectrophotometer (Thermo Scientific) and samples that had 260/280 ratios of 1.7 or below and/or 260/230 ratios of 1.8 or below were re-extracted. DNA integrity was assessed by electrophoresis on a 2% agarose gel.

Telomere length was measured using quantitative PCR (qPCR) on an Mx3005P (Stratagene). This method provides a relative measure of telomere length that is suitable for comparisons both within and among individuals of the same species. Telomere length was calculated as the ratio (T/S) of telomere repeat copy number (T) to control, single gene copy number (S) of the focal sample relative to a reference sample. We followed the methods of Cawthon 2002 adapted for European shags (Herborn *et al.* 2014). We used Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as the control, single copy gene and verified its suitability via a melt curve analysis, which demonstrated that the dissociation curve had a single peak. The melting temperature (Tm) is the temperature at which 50% of the DNA strands hybridise. This is sequence specific, and in this case the Tm is 79.7°C and indicated a single amplification product. To further verify that the amplification was a single product, we also ran a random selection of the amplified PCR products on a 2% agarose gel. All showed a single band at the expected size of 77bp.

Telomere and GAPDH reactions were carried out on separate plates. Each reaction was measured in triplicate and used 5 ng of DNA. The following primers were used to amplify the telomere and GAPDH sequences: Telomere *forward tel1b* (5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3') and *reverse tel2b* (5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'); Cormorant specific GAPDH *forward* (GACTGTAGCCTTCTCCTTCCCTTA) and *reverse* (TTCCCATCCACTTCCAGTAAAGA). The primer concentrations were: *forward tel1b/ reverse tel2b* 200 nM /200 nM and *forward GAPDH/ reverse GAPDH* 200 nM/ 200 nM. Primers were mixed with 12.5 µl of absolute blue SYBR green QPCR Master Mix (Stratagene) for a total volume of 25 µl. The conditions for the qPCR reactions were: *telomeres* 15 minutes at 95ºC,

followed by 27 cycles of 15 seconds at 95ºC, 30 seconds at 58ºC, and 30 seconds at 72ºC; *GAPDH* 15 minutes at 95ºC, followed by 40 cycles of 15 seconds at 95ºC, 30 seconds at 60ºC, and 30 seconds at 72ºC. In both reactions, the number of PCR cycles (Ct) necessary to accumulate sufficient fluorescent signal to cross a threshold was measured. Individuals with relatively long telomeres were characterized by shorter reaction times.

Each plate also contained a shag chick RBC reference sample that was serially diluted to produce a standard curve (10, 5, 2.5, 1.25, 0.75, and 0.375 ng) and to measure reaction efficiencies. All of the samples fell within the range of the standard curve. The average reaction efficiencies for the *GAPDH* (mean ± 1 SEM: 101.93% ± 0.90) and telomere plates (mean ± 1 SEM: 100.17% ± 1.37) were very close to 100%, and therefore we did not have to correct for efficiency differences when calculating telomere length. A 5 ng dilution of the reference sample was used to set the Ct thresholds for the reactions and to calculate the intra- and inter- plate variation.

To calculate the relative T/S ratios, we used the average Ct values for each sample according to the following formula: $2^{\Delta\Delta Ct}$, where $\Delta\Delta\ C_t = (C_t\ ^{telomere} - C_t\ ^{GAPDH})$ reference - $(C_t\ ^{telomere} - C_t\ ^{GAPDH})$ focal (Stratagene 2007). Average intra- and inter-plate variation of the C_t values was 1.02% and 1.13% for the telomere reactions and 0.70% and 0.73% for the *GAPDH* reactions. The inter-plate variation for the relative T/S ratio was 9.7%.

(c) Statistical analyses

We used linear mixed effects (LME) models to examine the potential influence of parental age on offspring initial telomere length (telomere length collected within the first two days of hatching) and the change in telomere length between sampling points (calculated as: the second telomere sample collected at the end of the linear growth period – the initial telomere sample). Variance structures were estimated using restricted maximum likelihood (REML) and all of the models had normal error structures.

We examined the potential effects of maternal and paternal age in different models as the age of the mother (age range = 2-19 years) was known in $n = 81$ nests for telomere length at hatching and $n = 64$ nests for the change in telomere length and the age of the father (age range = 2-22 years) was known in $n = 99$ nests for telomere length at hatching and $n = 80$ nests for the change in telomere length. In nests where the ages of both parents were known ($n = 129$ chicks

from n = 56 nests for telomere length at hatching and n = 96 chicks from n = 45 nests for the change in telomere length), the ages of the mother and father were highly correlated with one another (hatching: $r = 0.71$, $p < 0.001$, change in telomere length: $r = 0.73$, $p < 0.001$).

Models also included maternal and paternal identity as random factors to account for the non-independence of chicks produced in the same nest or by the same parent in different years (14 females and 24 males produced offspring in 2010 and again in 2011). In addition, we included year and sex as factors and clutch size, hatching order within the brood and hatch date (adjusted to the median for a given year) as covariates as these variables have been reported to influence offspring telomere dynamics in other studies (Hall *et al.* 2004; Foote *et al.* 2011; Nettle *et al.* 2013; Boonekamp *et al.* 2014).

In models of initial telomere length, we also included the actual ages of the chicks (all chicks were sampled within 2 days of hatching) and their mass at the time of sampling as this might be expected to influence telomere length. In models of the change in telomere length, we also included the linear growth rate (calculated as the slope of the regression of mass on age) as this has been reported to influence telomere loss in other studies (Geiger *et al.* 2012) and the number of days between the two sampling points. Telomere loss has been found to be greater in longer telomeres in some studies (Verhulst *et al.* 2013). However, after correcting for the regression to the mean (see Supporting Information) we did not find any relationship between initial telomere length and the change in telomere length.

We also examined whether maternal and paternal ages were correlated with clutch size, offspring mass at hatching, and offspring growth rate. Telomere length was natural log transformed to improve normality. Final models were determined using a backward elimination process and log-likelihood ratio tests. All statistical analyses were performed in IBM SPSS Statistics 19.

Results

There were no significant effects of maternal or paternal age on offspring initial telomere length (Table 1). In both maternal and paternal age models, chicks from larger clutches (Table 1, Fig. 1a and b), as well as chicks that were older and smaller had significantly shorter telomeres at the initial sampling point (Table 1). There were no significant effects of any of the other independent variables on initial telomere length (Table 1).

Chick telomere length significantly decreased between the initial sample collected within 2 days of hatching and the end of the linear growth period in both maternal ($F_{1, 129.72} = 8.669$, $p = 0.004$; initial telomere length: mean ± 1 SEM: 0.95 = ± 0.033, telomere length at the end of the linear growth period: mean ± 1 SEM: 0.86 = ± 0.030) and paternal age models ($F_{1, 159.02} = 11.695$, $p = 0.001$; initial telomere length: mean ± 1 SEM: 0.97 = ± 0.029, telomere length at the end of the linear growth period: mean ± 1 SEM: 0.88 = ± 0.027).

Importantly, in both maternal and paternal age models, there was a significant effect of the age of the parent on the change in offspring telomere length (Table 1). Chicks produced by older mothers and fathers experienced significantly greater telomere loss than chicks produced by younger parents (Table 1, Fig. 2a and 2b). None of the other independent variables were significantly related to the change in telomere length in either maternal or paternal age models (Table 1).

While telomere loss significantly decreased with parental age, the amount of loss was small relative to the amount of variation among chicks in the initial telomere length, and thus there was no significant effect of maternal ($F_{1, 60.53} = 0.172$, $p = 0.680$) or paternal age ($F_{1, 59.75} = 0.012$, $p = 0.912$) on chick telomere length at the end of the linear growth period.

There were no significant correlations between maternal or paternal age and clutch size ($r = -0.065$, $p = 0.440$; $r = 0.100$, $p = 0.202$), offspring mass at hatching ($r = 0.008$, $p = 0.921$; $r = -0.086$, $p = 0.272$), or offspring growth rate ($r = 0.088$, $p = 0.294$; $r = 0.116$, $p = 0.139$).

Discussion

In many organisms, parental age at conception has been shown to influence offspring lifespan and offspring telomere dynamics could be an important mechanism underlying this effect. In support of this, in European shags, although we found no effect of parental age on offspring telomere length collected within two days of hatching, we did find that chicks produced by older mothers and fathers had greater post-hatching telomere loss than those produced by younger parents. Because the ages of the parents were highly positively correlated, we did not have the statistical power to separate the relative influences of female and male age on offspring telomere dynamics and the ages of both pair members could have contributed to our results. However, our results seem to indicate that the maternal age effect is stronger than the paternal age effect.

This study also provides novel evidence that at least in this species, these effects are most likely the result of loss that occurred during the post-natal period rather than initial differences in telomere length. It is possible that there were also some carry-over effects of gamete or egg quality that were not manifested until the post-natal period. Recent data from bustard chicks produced by artificial insemination and reared without parental involvement suggest that gamete quality of older males and females is reduced, with the mother's age affecting chick mass at hatching, and the fathers age affecting growth soon after hatching (Preston *et al.* 2015). Teasing apart such effects with respect to telomere length would require artificial insemination and a cross-fostering design that was not possible in our study (Tissier, Williams & Criscuolo 2014; Preston *et al.* 2015). We did not however, find any correlation between parental age and hatching mass or chick growth in our study.

In birds, there is evidence that individuals with shorter telomeres (Heidinger *et al.* 2012) and faster rates of telomere loss (Boonekamp *et al.* 2014) during early life have reduced lifespans. Hence, our findings are consistent with the idea that parental age influences offspring longevity in part through its effects on offspring telomere loss. Although offspring produced by older parents experienced greater telomere loss between hatching and approximately 30 days post-hatching, the amount of loss was small relative to the variation in initial length, thus the effect of parental age on offspring telomere length at 30 days was not significant. However, parents continue to care for chicks until they are at least 50 days old and it is possible that by the time the chicks reached independence this effect would have been significant. The relative influence of telomere length versus loss rate on longevity is still poorly understood (Monaghan 2010). Telomere length might be more important if once telomeres become critically shortened, tissue function is directly impaired. Alternatively, telomere loss might have a larger effect if telomere shortening reflects an individual's susceptibility to stress and/or ability to repair stress-induced damage.

In shags, parental age might have influenced offspring post-natal telomere loss through many potential routes (Herborn *et al.* 2015). For example, older parents might produce offspring that are more sensitive to stressors or are less able to repair stress-induced damage. In this population of shags, we have previously found that chicks exposed to higher levels of stress during post-natal development are more stress responsive and experience greater telomere loss (Herborn *et al.* 2014). Chicks produced by older parents might experience a more stressful rearing environment if older parents are less able to provide care than younger parents. Alternatively, because this is a cross-sectional study, these effects could be due to differential parental investment strategies that are linked to parental longevity. For example, parents that live longer

might invest less in offspring. Identifying the mechanisms by which parental age influences offspring post-natal telomere loss is an important area of future research.

In addition, we found that telomere length at hatching was influenced by clutch size; chicks that hatched from larger clutches, had shorter telomeres at hatching. We did not measure egg parameters in our study, but this effect could have occurred because females that lay larger clutches produce smaller or lower quality eggs. In striped plateau lizards (*Sceloporus virgatus*) yolk antioxidant levels decrease with clutch size (Weiss *et al.* 2011). Telomere loss is accelerated by exposure to oxidative stress (von Zglinicki 2002) and embryos that develop in eggs with lower antioxidant levels may be at higher risk. A recent study in jackdaws (*Corvus monedula*) found that chicks that were reared in experimentally enlarged clutches experienced greater post-natal telomere loss than those reared in experimentally reduced clutches (Boonekamp *et al.* 2014). However, we did not find any effect of clutch size on the rate of post-natal telomere loss in shags.

In conclusion, there was no effect of parental age on offspring telomere length at hatching, suggesting that a pre-natal effect was unlikely to have occurred. However, chicks produced by older parents experienced significantly greater post-natal telomere loss than chicks produced by younger parents. These results are consistent with the idea that telomere dynamics might be an important mechanism underlying the commonly observed relationship between parental age and offspring lifespan. In species that provide extended post-natal care, effects occurring during the post-natal period might be particularly salient.

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Data Accessibility

Data deposited in the Dryad Digital Repository: http://doi.org/10.5061/dryad.9bs10, (Heidinger et al. 2016)

References

Asghar, M., Bensch, S., Tarka, M., Hansson, B. & Hasselquist, D. (2015) *Maternal and genetic factors determine early life telomere length*.

Asghar, M., Hasselquist, D., Hansson, B., Zehtindjiev, P., Westerdahl, H., Bensch, S. (2015) Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. *Science,* **347,** 436-438.

Aubert, G. & Lansdorp, P.M. (2008) Telomeres and aging. *Physiological Reviews,* **88,** 557-579.

Barrett, E.L.B., Burke, T.A., Hammers, M., Komdeur, J. & Richardson, D.S. (2013) Telomere length and dynamics predict mortality in a wild longitudinal study. *Molecular Ecology,* **22,** 249-259.

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.

Blackburn, E.H. (2005) Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. *Febs Letters,* **579,** 859-862.

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 B-Biological Sciences,* **281,** 7.

Bouwhuis, S., Charmantier, A., Verhulst, S. & Sheldon, B.C. (2010) Trans-generational effects on ageing in a wild bird population. *Journal of Evolutionary Biology,* **23,** 636-642.

Broer, L., Codd, V., Nyholt, D.R., Deelen, J., Mangino, M., Willemsen, G., Albrecht, E., Amin, N., Beekman, M., de Geus, E.J.C., Henders, A., Nelson, C.P., Steves, C.J., Wright, M.J., de Craen, A.J.M., Isaacs, A., Matthews, M., Moayyeri, A., Montgomery, G.W., Oostra, B.A., Vink, J.M., Spector, T.D., Slagboom, P.E., Martin, N.G., Samani, N.J., van Duijn, C.M. & Boomsma, D.I. (2013) Meta-analysis of telomere length in 19 713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. *European Journal of Human Genetics,* **21,** 1163-1168.

Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenko, A. & Kerber, R.A. (2003) Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet,* **361,** 393-395.

Clutton-Brock, T. (1991) *The evolution of parental care*. Princeton University Press, Princeton, N.J.

Daunt, F., Monaghan, P., Wanless, S., Harris, M.P. & Griffiths, R. (2001) Sons and daughters: age-specific differences in parental rearing capacities. *Functional Ecology,* **15,** 211-216.

Daunt, F., Wanless, S., Harris, M.P. & Monaghan, P. (1999) Experimental evidence that age-specific reproductive success is independent of environmental effects. *Proceedings of the Royal Society B-Biological Sciences,* **266,** 1489-1493.

De Meyer, T., Rietzschel, E.R., De Buyzere, M.L., De Bacquer, D., Van Criekinge, W., De Backer, G.G., Gillebert, T.C., Van Oostveldt, P., Bekaert, S. & Asklepios, I. (2007) Paternal age at birth is an important determinant of offspring telomere length. *Human Molecular Genetics,* **16,** 3097-3102.

Ducatez, S., Baguette, M., Stevens, V.M., Legrand, D. & Freville, H. (2012) Complex interactions between paternal and maternal effects: parental experience and age at reproduction affect fecundity and offspring performance in a butterfly. *Evolution,* **66,** 3558-3569.

Eisenberg, D.T.A., Hayes, M.G. & Kuzawa, C.W. (2012) Delayed paternal age of reproduction in humans is associated with longer telomeres across two generations of descendants. *Proceedings of the National Academy of Sciences of the United States of America,* **109,** 10251-10256.

Entringer, S., Epel, E.S., Kumsta, R., Lin, J., Hellhammer, D.H., Blackburn, E.H., Wust, S. & Wadhwa, P.D. (2011) Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. *Proceedings of the National Academy of Sciences of the United States of America,* **108,** E513-E518.

Entringer, S., Epel, E.S., Lin, J., Buss, C., Blackburn, E.H., Simhan, H.N. & Wadhwa, P.D. (2012) Prenatal programming of newborn and infant telomere length. *European Journal of Psychotraumatology,* **3,** 1.

Foote, C.G., Daunt, F., Gonzalez-Solis, J., Nasir, L., Phillips, R.A. & Monaghan, P. (2011) Individual state and survival prospects: age, sex, and telomere length in a long-lived seabird. *Behavioral Ecology,* **22,** 156-161.

Forslund, P. & Part, T. (1995) Age and reproduction in birds - hypotheses and tests. *Trends in Ecology & Evolution,* **10,** 374-378.

Fox, C.W., Bush, M.L. & Wallin, W.G. (2003) Maternal age affects offspring lifespan of the seed beetle, Callosobruchus maculatus. *Functional Ecology,* **17,** 811-820.

Froy, H., Phillips, R.A., Wood, A.G., Nussey, D.H. & Lewis, S. (2013) Age-related variation in reproductive traits in the wandering albatross: evidence for terminal improvement following senescence. *Ecology Letters,* **16,** 642-649.

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,** 1500-1510.

Gillespie, D.O.S., Russell, A.F. & Lummaa, V. (2013) The effect of maternal age and reproductive history on offspring survival and lifestime reproduction in preindustrial humans. *Evolution,* **67,** 1964-1974.

Hall, M.E., Nasir, L., Daunt, F., Gault, E.A., Croxall, J.P., Wanless, S. & Monaghan, P. (2004) Telomere loss in relation to age and early environment in long-lived birds. *Proceedings of the Royal Society B-Biological Sciences,* **271,** 1571-1576.

Haussmann, M.F. & Heidinger, B.J. (2015) Telomere dynamics may link stress exposure and ageing across generations. *Biology Letters*.

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.

Hayward, A.D., Mar, K.U., Lahdenpera, M. & Lummaa, V. (2014) Early reproductive investment, senescence and lifetime reproductive success in female Asian elephants. *Journal of Evolutionary Biology,* **27,** 772-783.

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 of the United States of America,* **109,** 1743-1748.

Heidinger, B.J., Herborn, K.A., Granroth-Wilding, H.M.V., Boner, W., Burthe, S., Newell, M., Wanless, S., Daunt, F., Monaghan, P. (2016). Date from: Parental age influences offspring telomere loss. Dryad Digital Repository: http://doi.org/10.5061/dryad.9bs10

Herborn, K.A., Daunt, F., Heidinger, B.J., Granroth-Wilding, H.M.V., Burthe, S.J., Newell, M.A. & Monaghan, P. (2015) Age, oxidative stress exposure and fitness in a long-lived seabird. *Functional Ecology*.

Herborn, K.A., Heidinger, B.J., Boner, W., Noguera, J.C., Adam, A., Daunt, F. & Monaghan, P. (2014) Stress exposure in early post-natal life reduces telomere length: an experimental demonstration in a long-lived seabird. *Proceedings of the Royal Society B-Biological Sciences,* **281,** 7.

Horn, T., Robertson, B.C., Will, M., Eason, D.K., Elliott, G.P. & Gemmell, N.J. (2011) Inheritance of Telomere Length in a Bird. *Plos One,* **6,** 5.

Keefe, D.L., Liu, L. & Marquard, K. (2007) Telomeres and meiosis in health and disease. *Cellular and Molecular Life Sciences,* **64,** 139-143.

Kimura, M., Cherkas, L.F., Kato, B.S., Demissie, S., Hjelmborg, J.B., Brimacombe, M., Cupples, A., Hunkin, J.L., Gardner, J.P., Lu, X.B., Cao, X.J., Sastrasinh, M., Province, M.A., Hunt, S.C., Christensen, K., Levy, D., Spector, T.D. & Aviv, A. (2008) Offspring's leukocyte telomere length, paternal age, and telomere elongation in sperm. *Plos Genetics,* **4,** 9.

Lansing, A.I. (1947) A Transmissible, Cumulative, and Reversible Factor in Aging. *Journals of Gerontology,* **2,** 228-239.

Monaghan, P. (2010) Telomeres and life histories. the long and the short of it. *Year in Evolutionary Biology* (eds C.D. Schlichting & T.A. Mousseau), pp. 130-142. Wiley-Blackwell, Malden.

Monaghan, P. & Haussmann, M.F. (2006) Do telomere dynamics link lifestyle and lifespan? *Trends in Ecology & Evolution,* **21,** 47-53.

Nettle, D., Monaghan, P., Boner, W., Gillespie, R. & Bateson, M. (2013) Bottom of the Heap: Having Heavier Competitors Accelerates Early-Life Telomere Loss in the European Starling, Sturnus vulgaris. *Plos One,* **8,** 8.

Njajou, O.T., Cawthon, R.M., Damcott, C.M., Wu, S.H., Ott, S., Garant, M.J., Blackburn, E.H., Mitchell, B.D., Shuldiner, A.R. & Hsueh, W.C. (2007) Telornere length is paternally inherited and is associated with parental lifespan. *Proceedings of the National Academy of Sciences of the United States of America,* **104,** 12135-12139.

Nussey, D.H., Baird, D., Barrett, E., Boner, W., Fairlie, J., Gemmell, N., Hartmann, N., Horn, T., Haussmann, M., Olsson, M., Turbill, C., Verhulst, S., Zahn, S. & Monaghan, P. (2014) Measuring telomere length and telomere dynamics in evolutionary biology and ecology. *Methods in Ecology and Evolution,* **5,** 299-310.

Nussey, D.H., Froy, H., Lemaitre, J.F., Gaillard, J.M. & Austad, S.N. (2013) Senescence in natural populations of animals: Widespread evidence and its implications for bio-gerontology. *Ageing Research Reviews,* **12,** 214-225.

Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T. & Blomqvist, D. (2011) Sex Differences in Sand Lizard Telomere Inheritance: Paternal Epigenetic Effects Increases Telomere Heritability and Offspring Survival. *Plos One,* **6,** 8.

Oro, D., Hernandez, N., Jover, L. & Genovart, M. (2014) From recruitment to senescence: food shapes the age-dependent pattern of breeding performance in a long-lived bird. *Ecology,* **95,** 446-457.

Potts, G.R. (1971) Moult in shag Phalacrocorax aristotelis, and ontogeny of staffelmauser.. *Ibis,* **113,** 298-&.

Potts, G.R., Coulson, J.C. & Deans, I.R. (1980) Population dynamics and breeding success of the shag, Phalacrocorax aristotelis, on Farne Islands, Northumberland. *Journal of Animal Ecology,* **49,** 465-484.

Prescott, J., Du, M., Wong, J.Y.Y., Han, J. & De Vivo, I. (2012) Paternal age at birth is associated with offspring leukocyte telomere length in the nurses' health study. *Human Reproduction,* **27,** 3622-3631.

Preston, B.T., Saint Jalme, M., Hingrat, Y., Lacroix, F. & Sorci, G. (2015) The sperm of aging male bustards retards their offspring's development. *Nature communications,* **6,** 6146.

Priest, N.K., Mackowiak, B. & Promislow, D.E.L. (2002) The role of parental age effects on the evolution of aging. *Evolution,* **56,** 927-935.

Rabon, D.R. (2014) Effects of age and experience on reproductive performance of captive red wolves (Canis rufus). *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* **92,** 251-258.

Reichert, S., Rojas, E.R., Zahn, S., Robin, J.P., Criscuolo, F. & Massemin, S. (2014) Maternal telomere length inheritance in the king penguin. *Heredity (Edinb)*.

Stratagene (2007) Introduction to quantitative PCR: methods and application. Stratagene.

Tarin, J.J., Gomez-Piquer, V., Rausell, F., Navarro, S., Hermenegildo, C. & Cano, A. (2005) Delayed motherhood decreases life expectancy of mouse offspring. *Biology of Reproduction,* **72,** 1336-1343.

Tissier, M.L., Williams, T.D. & Criscuolo, F. (2014) Maternal Effects Underlie Ageing Costs of Growth in the Zebra Finch (Taeniopygia guttata). *Plos One,* **9,** 8.

Unryn, B.M., Cook, L.S. & Riabowol, K.T. (2005) Paternal age is positively linked to telomere length of children. *Aging Cell,* **4,** 97-101.

Verhulst, S., Aviv, A., Benetos, A., Berenson, G.S. & Kark, J.D. (2013) Do leukocyte telomere length dynamics depend on baseline telomere length? An analysis that corrects for 'regression to the mean'. *European Journal of Epidemiology,* **28,** 859-866.

Voillemot, M., Hine, K., Zahn, S., Criscuolo, F., Gustafsson, L., Doligez, B. & Bize, P. (2012) Effects of brood size manipulation and common origin on phenotype and telomere length in nestling collared flycatchers. *Bmc Ecology,* **12,** 8.

von Zglinicki, T. (2002) Oxidative stress shortens telomeres. *Trends in Biochemical Sciences,* **27,** 339-344.

Wanless, S., Harris, M.P (1997) Phalacrocorax aristotelis Shag. *BWP Update*, pp. 3-13.

Weiss, S.L., Kennedy, E.A., Safran, R.J. & McGraw, K.J. (2011) Pterin-based ornamental coloration predicts yolk antioxidant levels in female striped plateau lizards (Sceloporus virgatus). *Journal of Animal Ecology,* **80,** 519-527.

Figure and table captions

Table 1. The effects of maternal and paternal age on offspring telomere dynamics (initial telomere length collected within the first two days of hatching and change in telomere length). Backward elimination and likelihood ratio tests were used to produce final models. Significant variables with $p < 0.05$ are indicated in bold. Final models were determined using a backward elimination process and log-likelihood ratio tests. Variables in italics were not included in the final models and in these cases P-values were obtained just prior to removal.

Fig. 1 The relationship between clutch size and natural log-transformed offspring initial telomere length (collected within the first two days of hatching) (T/S ratio) (mean ± s.e.m.) in European shag chicks for maternal (a) and paternal (b) age models.

Fig. 2 The relationship between the age of the mother (a) or father (b) and the change in the telomere length of their chicks during post-natal development (measurements collected approximately at hatching and 30 days post-hatching) in European shags. Greater loss is characterized by a more negative change in telomere length value.

Maternal age: (range 2-19 years)				
Initial telomere length: *n* = 170 chicks from *n* = 81 nests				
Variance	**Fixed effects**	**F**	**Estimate (s.e.)**	**P value**
Mother/residual	Clutch size	19.407	-2.968 (0.068)	**<0.001**
0.025/0.096	Chick age	4.568	-0.112 (0.052)	**0.034**
Father/ residual	Mass	4.071	0.009 (0.004)	**0.045**
0.008/0.096	Sex	1.063	0.055 (0.053)	0.304
	Year	0.817	0.060 (0.066)	0.368
	Hatch date	2.584	0.005 ((0.003)	0.111
	Hatch order	0.133	0.011 (0.030)	0.716
	Maternal age	0.006	0.007 (0.009)	0.939
Change in telomere length: *n* = 124 chicks from *n* = 64 nests				
Variance	**Fixed effects**	**F**	**Estimate (s.e.)**	**P value**
Mother/residual	Maternal age	4.829	-0.017 (0.008)	**0.032**
0.004/0.060	Clutch size	2.054	0.096 (0.0673)	0.155
Father/residual	Hatch order	1.919	-0.037 (0.028)	0.198
0.020/0.060	Sex	1.568	0.0618 (0.049)	0.213
	Year	2.896	-0.106 (0.062)	0.094
	Days between	0.001	<-0.001 (0.017)	0.971
	Growth rate	<0.001	<-0.001 (0.004)	0.990
	Hatch date	1.131	-0.003 (0.003)	0.292
Paternal age: (range 2-22 years)				
Initial telomere length: *n* = 204 chicks from *n* = 99 nests				
Variance	**Fixed effects**	**F**	**Estimate (s.e.)**	**P value**
Mother/residual	Clutch size	15.801	-0.229 (0.058)	**<0.001**

0.0196/0.1051	Chick age	8.379	-0.143 (0.050)	**0.004**
Father/residual	Mass	8.396	0.011 (0.004)	**0.004**
<0.001/0.1051	Hatch order	3.223	0.051 (0.029)	0.074
	Sex	2.890	0.083 (0.049)	0.091
	Year	1.247	0.066 (0.059)	0.266
	Hatch date	0.228	0.001 (0.003)	0.635
	Paternal age	0.621	0.005 (0.058)	0.432
Change in telomere length: *n* = 153 chicks from *n* = 80 nests				
Variance	**Fixed effects**	**F**	**Estimate (s.e.)**	**P value**
Mother/residual	Paternal age	4.758	-0.012 (0.006)	**0.031**
<0.001/0.092	Clutch size	2.083	0.078 (0.054)	0.151
Father/residual	Year	1.390	-0.058 (0.049)	0.240
<0.001/0.092	*Days between*	0.526	-0.011 (0.016)	0.469
	Growth rate	0.009	< 0.001 (0.004)	0.926
	Hatch date	0.051	< 0.001 (0.003)	0.822
	Hatch order	1.225	-0.034 (0.031)	0.270
	Sex	0.311	-0.028 (0.049)	0.578

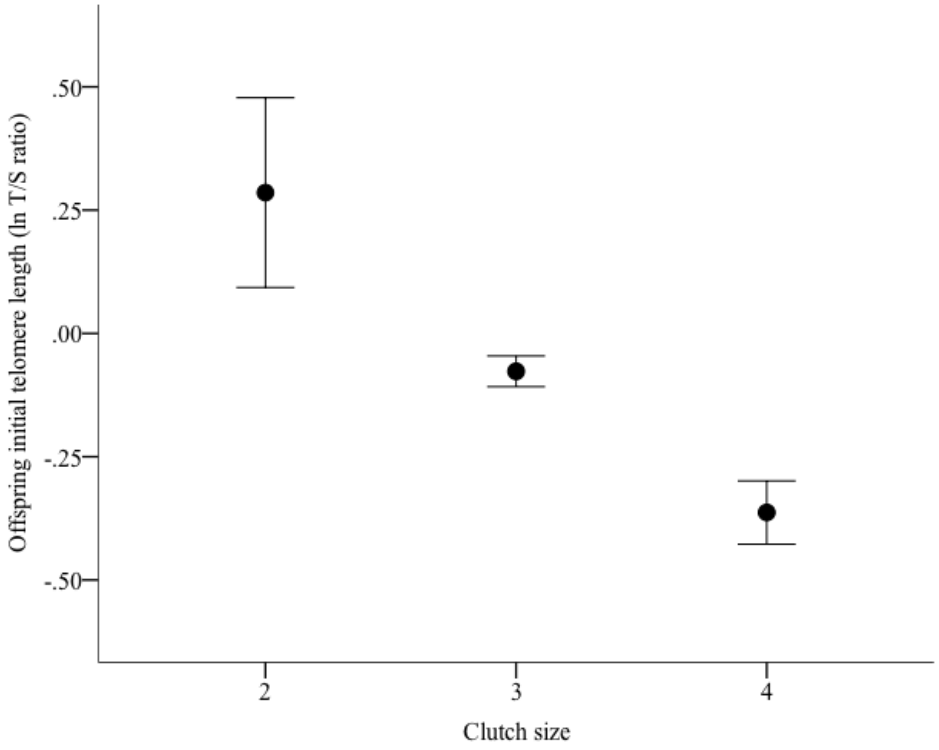

Fig. 1a.

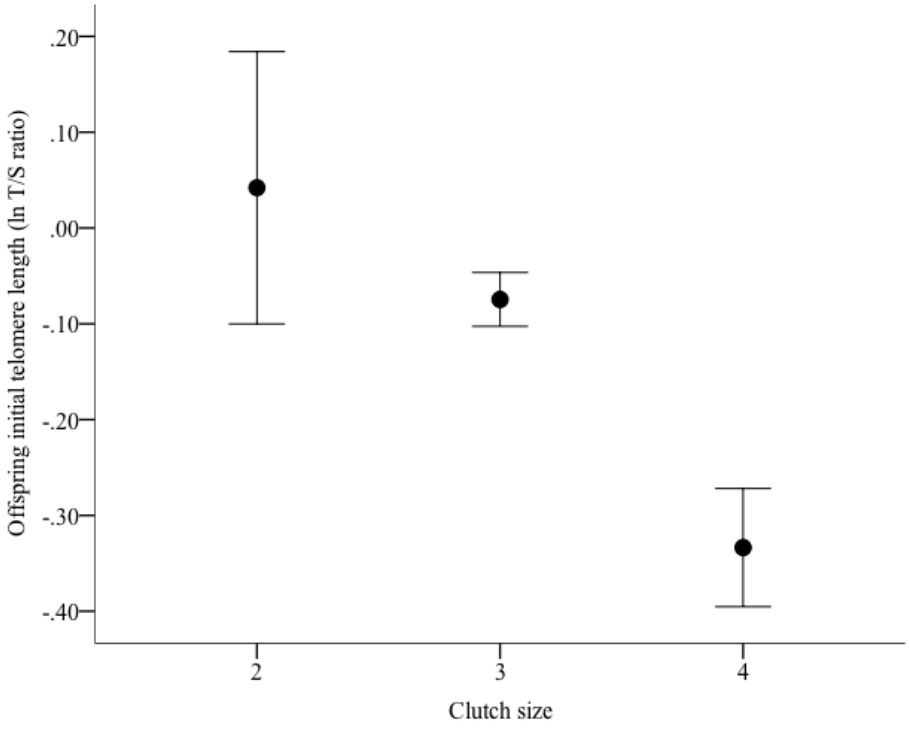

Fig. 1b.

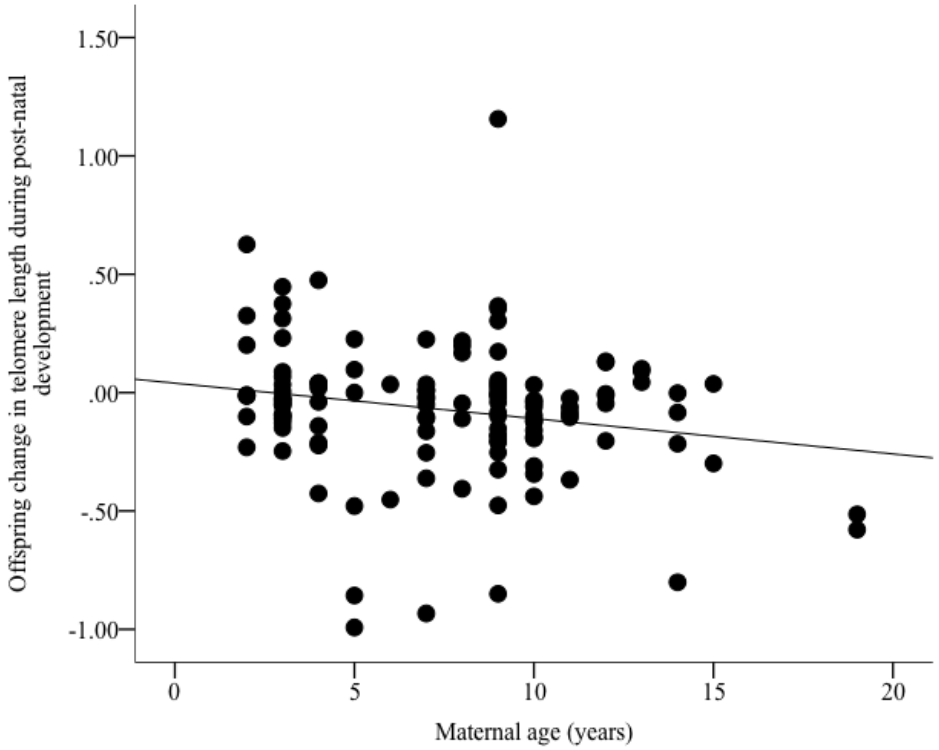

Fig. 2a.

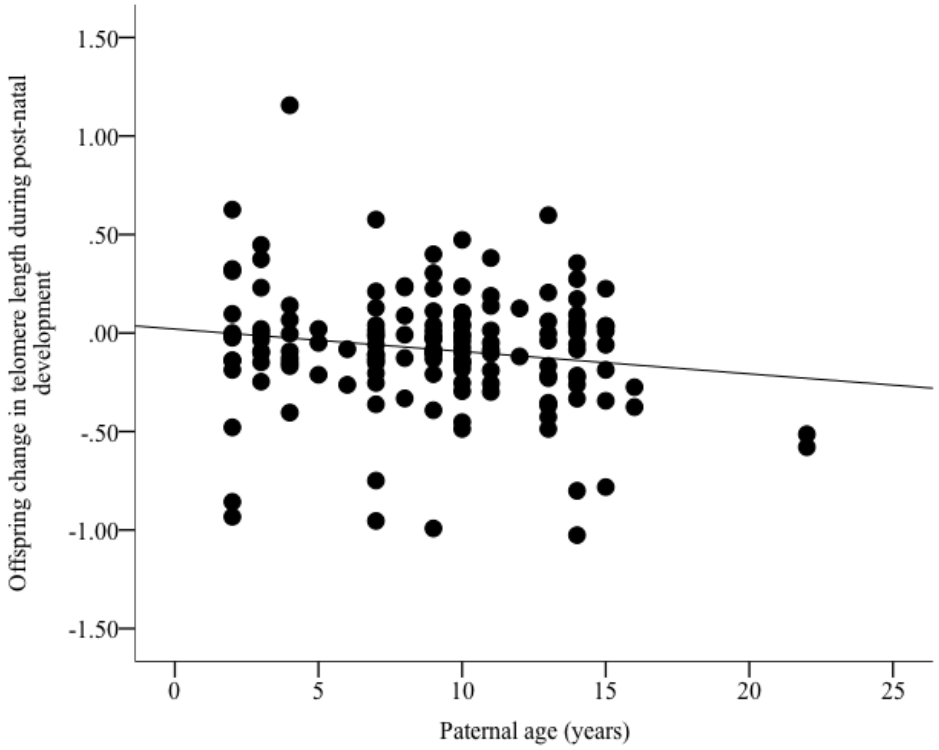

Fig. 2b.