Title: Evidence of paternal effects on telomere length increases in early-life

Running title: Paternal influence of telomere length

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Abstract:

Offspring of older parents in many species have decreased longevity, a faster ageing rate and lower fecundity than offspring born to younger parents. Biomarkers of ageing, such as telomeres, that tend to shorten as individuals age, may provide insight into the mechanisms of such parental age effects. Parental age may be associated with offspring telomere length either directly through inheritance of shortened telomeres or indirectly, for example through changes in parental care in older parents affecting offspring telomere length. Across the literature there is considerable variation in estimates of the heritability of telomere length, and in the direction and extent of parental age effects on telomere length. To address this, we experimentally tested how parental age is associated with the early-life telomere dynamics of offspring at two time points in a captive population of house sparrows *Passer domesticus*. Birds are an emerging model of ageing and telomere biology. We experimentally separated parental age from sex effects, and removed effects of age-assortative mating, by allowing the parent birds to only mate with young, or old partners. The effect of parental age was dependent on the sex of the parent and the offspring, and was found in the father-daughter relationship only. Older fathers produced daughters with a greater early-life increase in telomere length. Overall we found that offspring telomere length increased between the age of 0.5 and 3 months at the group and individual level. This finding is rare in birds with such increases more commonly associated with non-avian taxa. Our results suggest parental age effects on telomere length are sex-specific either through indirect or direct inheritance. The study of similar patterns in different species and taxa will help us further understand variation in telomere length and its evolution.

Key words: telomere dynamics, ageing, inter-generational effects, z-linked inheritance, transgenerational effects, Lansing effect

Introduction

Parent age at conception is often associated with their offspring's life-history, with offspring of older parents commonly having reduced reproductive success and longevity (Heidinger et al., 2016; Monaghan et al., 2020; Priest et al., 2002a; Schroeder et al., 2015), but see (Travers et al., 2021). Moreover, in some species, offspring of older parents experience higher rates of senescence, cellular ageing, and decreased longevity compared to their older siblings that may be associated with telomere attrition (Bouwhuis et al., 2010; Broer et al., 2013; Torres et al., 2011). While some studies did not find such cross-generational effects of age (Froy et al., 2017; Unryn et al., 2005), the cross-generational effects of age are reported across a wide range of taxa from rotifers (King, 1983) and insects (Priest et al., 2002a) to birds and mammals (Bize et al., 2009; Haussmann et al., 2003), and is termed the Lansing effect (Lansing, 1947).

One biomarker associated with biological age and longevity is the relative length of telomeres, the chromosome capping structures consisting of TTAGGG base pair repeats in vertebrates (Heidinger et al., 2012; Mather et al., 2011; Vedder et al., 2021). At each cell division telomeres shorten as the very ends of chromosomes are not replicated, known as the end-replication problem (Levy et al., 1992). Telomeres also partly function to prevent reactive oxygen species from damaging coding DNA and are damaged themselves in the process (Aubert and Lansdorp, 2008). The activity levels and expression of telomerase, the enzyme capable of elongating telomeres, decline rapidly in early life and are tissue specific (Taylor and Delany, 2000). Together these processes lead to a gradual telomere shortening over an individual's lifetime (Aubert and Lansdorp, 2008; Finkel and Holbrook, 2000), which is why telomere length has been investigated as a biomarker for biological age (Mather et al., 2011; Zglinicki and Martin-Ruiz, 2005). However, whether there is a direct causal link between telomere length and ageing remains unclear (Boonekamp et al., 2013; Simons, 2015).

In birds, telomere loss is fastest in early-life and an initially longer telomere length is associated with longer subsequent lifespans in captive and wild (Heidinger et al., 2016; Salomons et al., 2009; Wilbourn et al., 2018) bird populations. There is evidence for telomere length being heritable in birds (Vedder et al., 2021), and telomere dynamics have been associated with sex-specific parental age and telomere length (Asghar et al., 2015; Reichert et al., 2015). However, the direction of the association between telomere length, and maternal and paternal age varies even within bird species (Dugdale and Richardson, 2018; Heidinger and Young, 2020). In some bird species, the offspring of older mothers may have shorter telomeres and a faster attrition rate, especially in early development (Asghar et al., 2015). Conversely, negative associations between paternal age and offspring

telomere length have been observed in the absence of maternal correlation (Horn et al., 2011; Noguera et al., 2018; Sparks et al., 2021).

Between taxa, studies on the heritability of telomere length are conflicting. The heritability of telomere length can be sex-specific and is often larger in the heterogametic sex; suggesting some degree of maternal inheritance in birds (Asghar et al., 2015; Horn et al., 2011; Marasco et al., 2019; Reichert et al., 2015) and paternal inheritance in humans (Eisenberg et al., 2017; Njajou et al., 2007; Nordfjäll et al., 2009). However, homogametic inheritance of telomere length has been identified in humans (Broer et al., 2013), in birds (Bauch et al., 2019; Bouwhuis et al., 2018), and in lizards (Olsson et al., 2011). Furthermore, a lack of heritability has also been found in several bird species (Atema et al., 2015; Heidinger et al., 2012; Kucera, 2018). Overall, parental age effects on offspring telomere length, dynamics and heritability are complex, and vary in extent and direction within and between taxa.

Here, we test for sex-specific, age-related parental effects on offspring telomere length dynamics in captive house sparrows (*Passer domesticus*). By pairing different age categories of parents, we experimentally test the hypothesis that offspring of older parents have shorter telomeres and faster telomere attrition than offspring from younger parents.

Materials and Methods:

Study species and experimental design:

We used captive house sparrows at the Max Planck Institute for Ornithology, Seewiesen, Germany, during the breeding season of 2014. We used 42 pairs of male and female sparrows, which were assigned to four treatments, each with an equal sex ratio and a uniform distribution of ages across both sexes to control for age-assortative mating. We experimentally bred pairs in one of four age combinations: old-female/ old-male (OO, n=8 pairs), old-female/ young-male (OY, n=11 pairs), young-female/ old-male (YO, n=13 pairs), and young-female/ young-male (YY, n=10 pairs). Young birds hatched the preceding summer. Old (O) was defined as sparrows aged 4 years and older, although most individuals were 7 years or older (Males: 8 years = 2, 9 years = 21; Females: 4 years = 1, 7 years= 10, 8 years= 4, and 9 years= 1). The difference in age distribution between females and males corresponded to that observed in the wild, where females live shorter than males (Schroeder et al., 2012). We did not use the middle aged groups because in wild house sparrows, reproductive senescence may start at 3 years for females (Schroeder et al., 2012), or 5 years in males (Hsu et al. 2017). Each treatment group was split in two separate breeding groups located in separate aviaries. Each replicate aviary contained 15.3 (s.d= 4.9) males and 14.6 (s.d=2.4) females of the respective age class. Bird husbandry is described in Girndt et al. (2017).

Each replicate aviary was equipped with one more nest box than breeding pairs to reduce male-male competition for nest boxes. Sparrows were then allowed to naturally display, form pair bonds, choose a mate restricted by the age class present, and raise their young (Girndt et al., 2018). We systematically monitored breeding and identified the parents attending each nest box by observing the individual birds' colour ring combinations.

Blood sample collection:

We took blood samples from all chicks before they fledged, 0.5 months after they hatched (n=75). After fledging, offspring remained in the same aviary as their parents and siblings, and were blood sampled again (n=59) 2.5 months later. Blood samples were collected from the brachial vein of offspring using 1mm capillary tubes and stored in 1ml of 96% ethanol. We collected samples of 56 individuals at both 0.5 and 3 months to test for within-individual changes.

DNA extraction and quantification:

Following standard DNA extraction (Richardson et al., 2001), we measured the DNA concentration of the samples using a ThermoScientific NanoDrop8000 Spectrophotometer and standardised the concentration in our samples to 20-30ng/ml to ensure similar amplification of samples during qPCR. Where necessary, samples were diluted with T10E0.1 (10mM Tris-HCl, pH 8.0, 0.1mM EDTA, pH 8.0) or concentrated using a ThermoScientific Savant DNA SpeedVac Concentrator.

Estimation of telomere length:

We used multiplex qPCR to determine relative telomere length. We determined 'T' as the number of telomere repeats and 'S' as the number of control gene repeats. We then used the T/S ratio as a proxy for telomere length. The four DNA primers we used are described in Criscuolo et al. (2009). We used DNA from house sparrows not included in this analysis as a dilution standard at five DNA concentrations of approximately 80, 20, 5, 1.25 and 0.31ng/ml, on each plate. We then used these standards to produce a standard curve for all analysed samples. In each well we added 1.5µl of DNA sample, 0.9µl of each primer, 10µl of Sybr®Select Master Mix and 4.9µl ddH_2O. We ran each plate with an equal number of 0.5 and 3 months sample pairs from the same individual to account for any potential sample and plate effects when comparing within-individual changes in telomere length. We ran 42 samples, the five standards and a negative (with all components except a DNA sample) in duplicate on each 96-well plate. We ran the qPCR cycling conditions using QuantStudio 12kFlex Software v1.2.2 following the cycle timings given in Cawthon (2009). We analysed the software output to calculate the T/S ratio in each sample using a custom script that performed background subtraction, thresholding and standard curve correction (Appendix 1.1). We altered the thresholds for the standard curve of the telomere and GAPDH primers for each plate

based on amplification plots resulting in efficiencies of between 99.3-99.7 for GAPDH and 99.3-105.8 for telc and telg. The standard curve for each plate had an R^2 of 0.99 and the intra- and inter-plate variation coefficients all met adequate levels (Cawthon, 2009). We also ran a melt curve to confirm whether the expected two products were generated in the reaction. Additionally, we checked all plate amplification curves to see if DNA was present in the control, as this would indicate contamination. In all plates DNA was absent, apart from very late amplification due to primer dimerization. We repeated any sample duplicates that had a standard deviation of >0.05 following thresholding and used the mean T/S ratio of duplicates in our analysis. To test the reliability of these measurements we also calculated the repeatability of the T/S ratios at both time points using the individually duplicated T/S measurements, using the R package 'rptR' v.0.9.22 (Stoffel et al., 2017) with 1000 bootstrap iterations. Based on duplicates of the same sample the sample repeatability of the T/S ratios at 0.5 months was 0.98 (95% confidence interval: 0.97, 0.99), and at 3 months it was 0.99 (95% confidence interval: 0.99, 0.99). In our analyses, T/S ratios of offspring at 0.5 months old are referred to as $T/S_{0.5}$ and samples at 3 months old as T/S_3. We then calculated the difference between the two measurements as $\Delta T/S$. The within-individual repeatability of T/S ratios between the two time points was 0.27 (95% confidence interval: 0.01, 0.51). All samples were analysed for telomere length at the same time and had a similar shelf time (Lieshout et al., 2020). All reagents and equipment were produced by Thermo Fisher Scientific, Waltham, Massachusetts, US.

Ethical Note:

The Government of Upper Bavaria, Germany, approved the care, handling and husbandry of all birds in this study and granted a license for animal experiments to JS (Nr311.5–5682.1/1-2014-024).

Statistical Analysis:

We tested for a change in telomere length over the 2.5 months period by running a linear mixed effects model (LMM) with T/S as response variable, time of sampling (0.5 or 3 months) as a fixed effect, and individual chick ID as a random effect on the intercept. Next, we ran two further LMMs with the response variable $T/S_{0.5}$ and T/S_3, respectively. For each of these two models we tested the fixed effects of the paternal and maternal age categories (either 'young' or 'old' with 'old' as the reference level). To test for sex-specific parental effects, we included offspring sex as a fixed effect (with 'male' as the reference level) and an interaction of chick sex with parental age in the $T/S_{0.5}$ model. Because not all chicks were sampled at exactly 3 months after hatching (mean= 100.8 days, s.d.= 8.4), we also tested for an effect of the exact offspring age in days in T/S_3 using a LMM with T/S_3 as the response variable. There is a potential effect of the time a blood or DNA sample has

been stored until analysis (Sibma, 2021) and therefore we added 'sample age' as a fixed factor effect. We found that 'sample age' (a two-level categorical variable of either 'previously-' or 'newly-extracted') did not have a statistically significant effect on T/S_3 (posterior mode= -0.001, 95% credible interval, 95%CI= -0.01, 0.001, pMCMC= 0.81). Still, to account for any potential bias we retained 'sample age' as a fixed effect in the T/S_3 model. Note that our results however remained qualitatively similar whether or not we retained sample age in the model.

As the 0.5 months samples were also a mix of previously-, or newly-extracted DNA samples, we tested whether time of extraction had any effect on the calculated $T/S_{0.5}$ ratio as a result of DNA degradation (Madisen et al., 1987) (n samples newly-extracted= 10/75). We fitted a LMM with $T/S_{0.5}$ as the response and the time of extraction as a fixed effect, either 'newly-' or 'already extracted'. We found no statistically significant difference between newly- and already-extracted samples (posterior mode= -0.06, 95%CI= -0.20, 0.08, pMCMC=0.389). Further, a previous study investigating house sparrow telomere length found that the repeatability between newly- and previously-extracted samples was moderate (0.45, 95%CI= 0.35, 0.63; Sibma, 2021).

We included the nest box ID and aviary ID in which chicks were born as random effects on the intercept in all models to account for variance between broods and aviaries. We also included the random term of qPCR plate ID in all models to account for between-plate variance on the intercept. We examined the collinearity of the fixed effects, as collinearity could distort model results, but it did not exceed 0.7 (Dormann et al., 2013). All models were run using the Markov chain Monte Carlo (MCMC) method in the R package MCMCglmm v.2.29 (Hadfield, 2010).

Model validation:

As we used a Bayesian modelling approach, we deemed fixed effects to be statistically significant if their 95% credible intervals did not span zero, and we also report MCMC-p-values (pMCMC) (Hadfield, 2010). All terms were retained in models irrespective of their statistical significance. We directly assessed model autocorrelation for fixed and random effects to ensure that the risk of type I errors was not inflated. We also inspected iteration and density plots to ensure that effects showed equal variation around a constant mode and demonstrated convergence (Gelman and Hill, 2006; Hadfield, 2010). We ran all models for 100,000 iterations with a thinning interval of 10 and used minimally-informative flat priors. Gelman-Ruben statistics for all variables was between 1 and 1.05 indicating convergence (Brooks and Gelman, 1998). Effective sample sizes were >400 at all times and trace plots indicated good mixing of chains. All statistical analyses were carried out in R v.3.6.1 (R Core Team, 2021).

Results:

Unexpectedly, the telomere length of 80% of the offspring for which both measurements were available increased between 0.5 and 3 months of age (n=45/56; Fig. 1,Table 1).

We did not find a statistically significant effect of parental age class on T/S at 0.5 months, which is shortly before sparrows gain independence and fledge from their nest (Table 2). However, we detected statistically significant effects of paternal age on T/S at 3 months such that daughters of young fathers had shorter telomeres than daughters of old fathers (Table 2, Fig. 2). In contrast, paternal age had no statistically significant effect on the telomere length of sons at three months (Table 2, Fig. 2).

Discussion:

Individual chick telomere length increased between 0.5 and 3 months of age. This increase disagrees with much of the published literature, which generally finds a decrease in telomere length in early-life (Boonekamp et al., 2014; Cerchiara et al., 2017; De Meyer et al., 2007; Hoelzl et al., 2016; Salomons et al., 2009). While a population level increase in telomere length has previously been found in some long-lived bird species (Haussmann et al., 2007; Pauliny et al., 2012), other studies have found that telomeres elongation for a proportion of chicks is more common in shorter-lived species (A. M. Brown et al., 2021; T. Brown et al., 2021; Eisenberg, 2019) and few longer-lived species (Cerchiara et al., 2017). For example, a study on jackdaws *Corvus monedula* found that between 5 and 30 days post-hatching, telomere lengths increased for 25% of sampled offspring (Grasman et al., 2011). An increase in early-life telomere length has also been observed in non-avian taxa, including water pythons *Liasis fuscus* (Ujvari and Madsen, 2009) and European badgers *Meles meles* (Lieshout et al., 2019). A lack of comparable published research exploring a change in telomere length using multiple time points in early life may, in part, explain the surprising nature of our observed increase in telomere length in early-life.

An increase in telomere length can have methodological (Sheldon et al., 2021) and/or biological explanations (Ujvari and Madsen, 2009). First, it could be due to DNA in samples degrading over time (Madisen et al., 1987; but see Seutin et al., 1991). Since we used previously-extracted DNA for the majority of 0.5 month samples, we investigated whether differential telomere degradation rates between DNA and blood sample extraction types could be a cause for the observed increase. We found no statistically significant difference between the telomere lengths of newly- and previously-extracted samples and thus, telomere degradation in extracted samples over time seems like an unlikely explanation for our results.

Second, qPCR plates contained both 0.5 and 3 months samples, and between-plate variance was negligible in all our models, highlighting that this element of our methodology had little impact on our results. Overall, we monitored procedural efficiency throughout data collection and did not identify any other potential methodological sources of variation; the repeatability estimates for the T/S ratios estimated from within-individual samples were well within the range of those for similar species in other qPCR studies (Kärkkäinen et al., 2021). Consequently, we believe that the increase in telomere length observed in our study has a biological explanation. For example, telomerase activity might have been maintained in the offspring after the first sample was taken. Indeed, two studies have shown that telomerase activity can be maintained up to five weeks post-hatching in zebra finches *Taeniopygia guttata* (Haussmann et al., 2007) and chickens *Gallus gallus* (Taylor and Delany, 2000). Yet, neither of these studies assessed telomerase activity at multiple time points in the same individual's early-life post-hatching, which remains as an interesting future avenue for the field.

While we expected that old parents would produce offspring with shorter telomeres, as found in other short-lived bird species in line with predictions of the Lansing effect (Bauch et al., 2019; Criscuolo et al., 2017), our experimental approach found that old fathers produced daughters with longer telomeres, but only 3 months after hatching, potentially indicating an environmental or an age-dependent epigenetic effect. Positive relationships between parental age and offspring telomere length have previously been found previously in long- and short-lived bird species (Asghar et al., 2015; Becker et al., 2015; A. M. Brown et al., 2021; Dupont et al., 2018). Such effects may arise indirectly for example through improved parental care that older individuals provide compared to inexperienced, young breeders. However the positive effect of improved parental care by older parents also shows senescence in the oldest individuals (Beamonte-Barrientos et al., 2010). It is also possible that these effects arise from a phenomenon whereby parents that confer longer telomere length to their young also survive for longer. These positive effects of parnetal age on telomere length are inconstrast to some previous studies that found a negative effect of parental age resulting from the poorer condition of these old individuals (Bouwhuis et al., 2018; Criscuolo et al., 2017), or a lack of an effect of parental age on parental care (Nakagawa et al., 2007). Further, in some studies testing parent sex-specific effects, offspring telomere length was found to correlate only with maternal age, and only relatively soon after hatching (ten days in king penguins *Aptenodytes patagonicus*: Reichert et al. (2015); nine days in great reed warblers *Acrocephalus arundinaceus*: Asghar et al. (2015)). As we found no effect of maternal age in our study, an influence of maternal age on offspring telomere length may well have been present, but already diminished below detectable levels 0.5 months after hatching, when our first sampling took place.

While telomere lengths in offspring have been shown to be affected by an offspring's environment (Dugdale and Richardson, 2018; Lieshout et al., 2021), effects of paternal age in birds have been found to be independent of this (Bauch et al., 2019; Boonekamp et al., 2014). As such, there is overall growing support for at least contributory paternal inheritance of telomere length in some species of birds (Bouwhuis et al., 2018; Olsson et al., 2011; this study). The combined positive effect of having an older father has been theorised to result from an upregulation of telomerase activity in sperm and a subsequent increase in gamete telomere length as males age (De Meyer et al., 2007). Therefore, one possibility is that a combination of telomerase activity in sperm in fathers, a form of Z-linked inheritance, and potential parental care benefits (discussed above) may explain the positive effect of increasing father age on offspring telomere lengths, with larger effects seen on daughters compared to sons as observed here.

However, we did not detect an effect of parental age on offspring telomere length at 0.5 months after hatching. This is again in contrast to studies finding evidence to support the Lansing effect where offspring of older parents may have shorter telomeres in early-life with potential implications for longevity (Monaghan et al., 2020; Priest et al., 2002b). Heidinger et al. (2016) similarly found no effect of parental age on offspring telomere length in early-life at 25 days after hatching in European shags *Phalacrocorax aristotelis*. Further, variation in pre-fledging telomere length may in part be explained by brood-specific additive genetic effects (Voillemot et al., 2012). As such, it may be that at later time points effects of parent age and post-fledging environmental factors appear to be more important than brood-specific effects in determining offspring telomere length. Again, there is a need for more studies investigating the relationship between paternal age and telomere dynamics to detect when and how patterns of telomere dynamics are driven.

In summary, we find that telomere lengths increased in early-life with a likely biological cause. Furthermore, our results indicate that paternal age effects are more influential on offspring telomere length than maternal age effects in our population of house sparrows, with the daughters of older fathers having longer telomeres. Future analyses of telomerase activity levels in both the sperm of adult males and the somatic tissues of offspring would yield further insights into the drivers of parental age effects on offspring telomere dynamics in early-life.

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Competing interests' statement:

No competing interests declared.

Author contributions:

Conceptualization: SB, JS; Methodology: SB, JS, MJPS; Validation: SB, MJPS; Formal analysis: SB, JS; Investigation: SB, AG, JS, AST; Resources: JS, MJPS, TB; Data curation: SB, JS, TB; Writing- original draft: SB; Writing- review & editing: SB, JS, AG, AST, MJPS; Visualization: SB, JS, AG, AST; Supervision: JS; Project administration: JS, TB; Funding acquisition: JS, TB.

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Data availability statement:

The data and code are available at the Open Science Foundation through this link: https://osf.io/6kwzh/?view_only=96b0d8a81ce84ba09b364e514ab0072e.

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Table 1: Results from a Bayesian MCMC linear mixed-effects model testing the difference between telomere length in house sparrow chicks at 0.5 and 3 months of age.

Parameter	*Estimate*	*95% credible intervals*	p_{MCMC}
Intercept	**0.93**	**0.84 - 0.99**	**<0.001**
Chick age	**0.19**	**0.12 - 0.26**	**<0.001**
Random effects			
Chick ID	0.00	0.00 - 0.01	
Nest box ID	0.02	0.01 - 0.04	
Aviary ID	0.00	0.00 - 0.00	
qPCR plate ID	0.00	0.00 - 0.01	
Residual	0.04	0.02 - 0.05	

Chick age was modelled as either 0.5 months (75 chicks) or 3 months (59 chicks), with 0.5 months as a reference level. Estimates shown are posterior modes. Statistically significant effects are shown in bold.

Table 2: Results from two Bayesian MCMC linear mixed-effects models with telomere length of house sparrow chicks at age 0.5 months ($T/S_{0.5}$) and 3 months (T/S_3) as response variables, respectively.

	$T/S_{0.5}$			T/S_3		
Parameter	*Estimate*	*95%CI*	p_{MCMC}	*Estimate*	*95%CI*	p_{MCMC}
Intercept	**0.97**	**0.84 - 1.10**	**<0.001**	**1.57**	**0.32 – 2.71**	**0.022**
Chick sex	-0.08	-0.22 - 0.11	0.469	-0.23	-0.41 - 0.61	0.121
Maternal age	-0.07	-0.23 - 0.08	0.346	0.06	-0.32 - 0.21	0.711
Maternal age x Chick sex	0.06	-0.15 - 0.25	0.597	0.09	-0.21 - 0.43	0.573
Paternal age	-0.03	-0.20 - 0.10	0.481	**-0.27**	**-0.52 - 0.00**	**0.047**
Paternal age x Chick sex	0.6	-0.07 - 0.34	0.168	**-0.40**	**0.09 - 0.71**	**0.016**
Sample day				0.00	-0.01 - 0.01	0.694
Random effects						
Nest box ID	0.01	0.00 - 0.03		0.01	0.00 - 0.02	
Aviary ID	0.00	0.00 - 0.01		0.01	0.00 - 0.01	
qPCR plate ID	0.00	0.00 - 0.01		0.00	0.00 - 0.01	
Residual	0.08	0.03 - 0.11		0.08	0.03 - 0.11	

Maternal and paternal age were modelled as either young or old; young was <2 years old, and old was determined as >3 years old). 0.5 months: n= 69 chicks, 3 months: n= 59. The reference level for parental ages was 'old', and 'female' was the reference level for chick sex. Estimates shown are posterior modes. Statistically significant effects are shown in bold.

Figure legends:

Figure 1: Change in telomere length (log(T/S Ratio)) within house sparrow chicks at 0.5 and 3 months of age. A) Individuals are connected by a line (n offspring with samples at 0.5 months= 75, at 3 months= 59). B) Boxplots show the mean (central line) and 25th and 75th percentiles (lower and upper box bounds respectively) of the log(T/S Ratio) within age group of the chicks' parents (Y = 3 years old). T/S Ratio is presented on the log scale to aid visualisation. YO = young mothers, old fathers (n= 19, 12). OO = both parents old (n= 18, 19). OY = old mothers, young fathers (n= 17, 18). YY = both parents young (n= 15, 10).

Figure 2: Daughters of 'old' fathers had statistically significantly longer telomeres. Posterior modes (red and blue dots) and corresponding 95% credible intervals (lines) from a linear mixed-effects model testing the relationship between T/S_3, father age, and sex of chicks (Table 1). Fathers were assigned an age category of young, 'Y', or old, 'O'. A young father was <2 years old, and an old father was determined as >7 years old. Chick sex is indicated as either female, 'red', or male, 'blue'. The number of offspring in each category; Y, and female= 12, male= 16, O, and female= 16, male= 15. Raw data points are shown as grey dots.