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

Running title: Paternal influence of telomere length

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Summary statement: We experimentally demonstrate that older fathers produced daughters with a greater early-life increase in telomere length in a captive population of house sparrows *Passer domesticus*

Abstract:

Offspring of older parents in many species display decreased longevity, a faster ageing rate and lower fecundity than offspring born to younger parents. Biomarkers, including telomeres, that tend to shorten as individuals age, may provide insights into the mechanisms of parental age effects. Parental age could associated with telomere length either through inheritance of shortened telomeres or through indirect effects, such as variation in parental care with parental ages, which leads to variation in offspring telomere length. Howeverthere is considerable variation across studies quantifying the the heritability of telomere length, and the direction and extent of parental age effects. To address this, here we experimentally investigate how parental age is associated with the earli-life telomere dynamics of offspring at two time points in a captive population of house sparrows (*Passer domesticus*). We experimentally separated parental age from sex effects, and from effects stemming from age-assortative mating by allowing the parents to only mate with young, or old partners. We found that telomere length of the offspring increased between the age of 0.5 and 3 months at the group and individual level, which has been reported previously predominantly in non-avian taxa. We further show that older fathers produced daughters with a greater early-life increase in telomere length, indicating support for a potential sex-specific inheritance, and for sex-specific non-genetic effects of parental age on early-life telomere length. Overall, our results highlight the need for more studies testing early-life telomere dynamics and sex-specific heritability of telomere length.

Introduction

Parental 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., 2002; 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 (Bouwhuis et al., 2010; Broer et al., 2013; Torres et al., 2011). While some studies did not find such effects (Froy et al., 2017; Unryn et al., 2005), the associations are reported across a wide range of taxa from rotifers (King, 1983) and insects (Priest et al., 2002)to birds and mammals (Bize et al., 2009; Haussmann et al., 2003) and is termed the Lansing effect (Lansing, 1947)

The relative length of telomeres, the chromosome capping structures consisting of TTAGGG base pair repeats in vertebrates, is associated with biological age and longevity (Heidinger et al., 2012; Mather et al., 2011; Vedder et al., 2021). Telomeres partly function to prevent DNA damage from reactive oxygen species (Aubert and Lansdorp, 2008). The activity levels of telomerase, the RNA-protein complex responsible for ligating TTAGGG repeats, decline rapidly in early life and are tissue specific (Taylor and Delany, 2000). Together this leads to a gradual telomere shortening over an individual's lifetime (Aubert and Lansdorp, 2008; Finkel and Holbrook, 2000), which is why telomere length is often used 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 an individual's age 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 lifespan in captive (Reichert et al., 2013; Wilbourn et al., 2018) and wild bird populations (Haussmann et al., 2003; Heidinger et al., 2016; Reed et al., 2008; Richardson et al., 2001; Salomons et al., 2009; Vedder et al., 2021). 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; Horn et al., 2011; Reichert et al., 2015; Salomons et al., 2009). This suggests that indeed, some Lansing-type effects may be inherited via telomere length. 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). The offspring of older mothers may have shorter telomeres and a faster attrition rate, especially in early development (Asghar et al., 2015; Salomons et al., 2009). 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 also been found in humans (Broer et al., 2013), in some bird species (Bauch et al., 2019; Bouwhuis et al., 2018) and in lizards (Olsson et al., 2011). Furthermore, a sex-specific 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 of impact within and between taxa.

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

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 (Y) 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 male (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 2.5 months later were blood sampled again (n=59). 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 standards at five DNA concentrations of 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 old 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 (Appendix 1.1). We altered the thresholds for the standard curve of the telomere and GAPDH primers for each plate to optimise amplification efficiencies to between a standard of 95 and 110. Efficiencies for each plate were 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 examine 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, or present only in very low levels in negatives, 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' (Stoffel et al., 2017) with 1000 bootstrap iterations. The repeatability of the T/S ratios at 0.5 months was 0.98 (95% credible interval, CI: 0.97, 0.99), and at 3 months it was 0.99 (95%CI: 0.99, 0.99). T/S ratios of offspring at 0.5 months old are referred to as $T/S_{0.5}$ and samples at 3 months old T/S_3 in our analyses. We then calculated the difference between the two measurements as $\Delta T/S$. The repeatability of T/S ratios between the two time points was 0.27 (95% CI: 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 an explanatory fixed factor, 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 model 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 categorical variable (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 sampples has been stored until analysis (Sibma, 2021) and therefore we added 'sample age' as an explanatory covariate. We found that 'sample age' (a two-level categorical variable of either 'previously-' or 'newly-extractled') did not have a statistically significant effect on T/S_3 (posterior

mode= -0.001, 95% credible interval= -0.01, 0.001, pMCMC=0.809). 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 a mix of newly- and already-extracted DNA samples, we also tested whether time of extraction had any effect on the calculated T/S ratio as a result of DNA degradation (Madisen et al., 1987) (n samples newly-extracted= 10 out of 75). We fitted a LMM with $T/S_{0.5}$ as the response and the time of extraction as a fixed factor, either 'newly-' or 'already-extracted'. We found no statistically significant difference between newly- and already-extracted samples (posterior mode= -0.06, 95% credible interval= -0.20, 0.08, pMCMC=0.389). Further, a previous study investigating house sparrow telomere length found that the repeatability between freshly- and previously-extracted samples was moderate (0.45, 95%CI= 0.35, 0.63). 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)

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. 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 terms to be statistically significant if their 95% credible intervals (95CI) 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 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 default priors, these settings allowed for good mixing of the chain. All statistical analyses were carried out in R v.3.6.1 (R Core Team, 2019).

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; Figure 1 and Table 1).

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% confidence 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	0.02	0.01 - 0.04	
Aviary	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 a binary variable of either 0.5 months or 3 months, with 0.5 months as a reference level. 0.5 months: n= 75 chicks, 3 months: n= 59. Estimates shown are posterior modes. Statistically significant effects are shown in bold.

We did not find a statistically significant effect of parental age class on $T/S_{0.5}$, which is shortly before sparrows gain independence and fledge from their nest (Table 2). However, in the T/S_3 model we detected statistically significant effects of paternal age 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).

Table 2: Results from two Bayesian MCMC general linear mixed-effects models with telomere length of house sparrow chicks at age 0.5 months and 3 months as response variable, 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	0.01	0.00 - 0.03		0.01	0.00 - 0.02	
Aviary	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 a binary variable of either young or old; young was <2 years old, and old was determined as >3 years old for females and >7 years for males). 0.5 months: n= 69 chicks, 3 months: n= 59. The reference level for parental ages was 'old', and the 'female' was the reference level for chick sex. Estimates shown are posterior modes. Statistically significant effects are shown in bold.

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 smaller, shorter-lived species (Brown et al., 2021a; Brown et al., 2021b; 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 length 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 and/or biological explanations. 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 pre-extracted DNA for the majority of 0.5 month samples, we investigated whether differential telomere degradation rates between extracted DNA and blood sample types could be a cause for the observed increase. However, we found no statistically significant difference between the telomere length of newly- and already-extracted samples and thus, telomere degradation in samples over time is 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, and so, we are convinced 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. A further explanation may be that the increase we find in offspring telomere length is partly driven by the methodology we used to quantify telomere length. Studies where telomere length is determined via qPCR, as in this study, also quantify interstitial telomeres, in contrast to Telomere Restriction Fragment analysis (TRF) studies which only quantify telomeres at chromosome caps. Consequently studies using the qPCR method may be more likely to identify initially longer telomere length, though these should also identify the same relative changes in length over an individual's lifetime.

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. Similar positive effects of parental age have also previously been found in long- and short-lived bird species (Asghar et al., 2015; Becker et al., 2015; Brown et al., 2021a, 20; Dupont et al., 2018). Positive effects of parental age on offspring telomere length may arise from a potentially improved parental care that older individuals may be able to provide compared to inexperienced, young breeders, though this effect also shows senescence (Beamonte-Barrientos et al., 2010). It is also possible that these effects may simply arise from a phenomenon whereby parents that confer longer telomere length to their young also survive for longer. Again though, previous studies have 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: Reichert et al., 2015; nine days: Asghar et al., 2015). As we found no influence 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.

While telomere length 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, overall, there is 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, 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 length, 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., 2002). Heidinger et al., 2016 similarly found no effect of parental age on offspring telomere length in very 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 parental 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 sum, 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, TB; 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. Upon acceptance of this work the data and code will be permanently hosted in a public repository and their DOI provided.

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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 = <2 years old, O = >3 years old for females and >7 years old for males). 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). Chick telomere lengths differed between time points, Bayesian MCMC linear mixed-effects model, $p<0.001$.

Figure 2: Post-hoc effect size plot from a linear mixed-effects model testing the relationship between T/S$_3$, father age, and sex of chicks (Table 2). 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. Squares represent the model estimated effect sizes of T/S ratio for each paternal age x chick sex, associated lines represent 95% credible intervals. Daughters of 'old' fathers had significantly longer telomeres, Bayesian MCMC linear mixed-effects model, $p=0.016$.