**Offspring telomere length in the long lived Alpine swift is negatively related to the age of their biological father and foster mother**

François Criscuolo1‡$, Sandrine Zahn1$ and Pierre Bize2‡

1Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, France

**2**University of Aberdeen, School of Biological Sciences, Aberdeen, AB24 2TZ, United Kingdom

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‡Correspondence: francois.criscuolo@iphc.cnrs.fr; pierre.bize@abdn.ac.uk

$ These authors share a first co-authorship

**Abstract**

A growing body of studies is showing that offspring telomere length (TL) can be influenced by the age of their parents. Such a relationship might be explained by variation in TL at conception (gamete effect) and/or by alteration of early growth conditions in species providing parental care. In a long-lived bird with bi-parental care, the Alpine swift (*Apus melba*), we exchanged an uneven number of 2 to 4-day-old nestlings between pairs as part of a brood size manipulation. Nestling TL was measured at 50 days after hatching, which allowed investigating the influence of the age of both their biological and foster parents on offspring TL, after controlling for the manipulation. Nestling TL was negatively related to the age of their biological father and foster mother. Nestling TL did not differ between enlarged and reduced broods. These findings suggest that offspring from older males were fertilised by gametes with shorter telomeres, presumably due to a greater cell division history or a longer accumulation of damage, and that older females may have provided poorer parental care to their offspring.

**Introduction**

Telomeres are repeated non-coding DNA sequences protecting the end of linear chromosomes [1]. Telomeres shorten with each cell division because the DNA polymerase does not replicate in full the end of the chromosomes: as a consequence, telomere length has been associated with chronological ageing of the whole organism [2]. In addition, stress triggers telomere loss due to its deregulating impacts on the mechanisms stabilizing telomere length [3]. The stress–telomere relationship has received increasing attention because both parameters are related to individual health and survival prospects and because intergenerational shortening of telomere length may exist [4]. Interestingly, a growing body of studies has shown that offspring telomere length (TL) is negatively (but see [5]) related to the age of their parents but such link has only been tested in a few species [6, 7] and the underlying mechanisms remain evasive. Such a relationship might be explained by variation in TL at conception (gamete effect) and/or by alteration of early growth conditions in the offspring of species providing parental care.

Parental age may negatively influence offspring TL, if older parents produce gametes with shorter telomeres [8]. Remarkably, because telomere shortening can be prevented or telomeres can even be elongated by the enzyme telomerase, and because telomerase is thought to be robustly expressed in the male germinal tissues, it has also been proposed that older fathers may produce gametes with longer telomeres and, in turn, father offspring with longer telomeres [9].

A second set of hypotheses focusses on age-related variation in parental care and emphasises the post-birth environment as an important mediator of offspring TL [10] (but see [11] for pre-birth effets). Because parenting experience should improve with age, younger parents may provide a lower quality environment during the growth of their offspring [12], which in turn may hasten telomere shortening. Alternatively, older parents may become less capable of providing a high quality environment and, thus, their offspring may end up with shorter telomeres at independence.

Our study tested two specific questions in the long-lived Alpine swift (*Apus melba*): (i) is there a relationship between parental age and offspring TL? (ii) based on a cross-fostering design, we investigated whether offspring TL was best explained by the age of their biological parents (i.e. gamete effect) and/or by the age of their foster parents (i.e. parental care effect).

**Material and Methods**

*General procedures*

Data were collected in 2004 and 2006 in a colony of ~100 breeding pairs of swifts in Bienne, Switzerland. When nestlings were 2-4 days of age, we performed a brood size manipulation by exchanging an uneven number of nestlings (usually 1 chick against 2) between pairs of nests (14 in 2004 and 15 in 2006) with a similar clutch size, hatching date and brood size at hatching. Parents were ranging from to 2 to 15 years of age (see [13]). Brood size at 50 days after hatching in reduced and enlarged broods was 1.3 ± 0.1 and 2.3 ± 0.1 chicks, respectively (Wilcoxon test: *z* = -5.02, *P* < 0.001).

At the age of 50-days (one week before fledging), blood samples were taken for telomere analysis, while body mass, wing length, and sternum length were measured. Whole blood was collected in EDTA tubes and stored at -80°C. Adults were captured on their eggs or soon after chicks hatched. Because nestlings have been ringed each year since 1968 and adults have been the subject of an individual-based study since 2000, we assigned the age of adults either based on their year of ringing as a nestling (36 of 54 males and 44 of 54 females) or based on their first appearance as breeders.

*Relative telomere length (RTL) measurement*

Genomic DNA was extracted from nucleated red blood cells using the DNeasy Blood and Tissue Kit (Qiagen). RTL was measured in 95 chicks, using a multiplex quantitative PCR (qPCR) (see Electronic Supplementary Material, ESM 1).

*Statistical analyses*

Nestling traits were analysed in JMP 11.0 (SAS Institute Inc) using linear mixed models with the nests of origin and of rearing as two random factors, brood size manipulation as a fixed factor, and the age of the genetic and foster parents as four cofactors. The age of the parents were not correlated between and within broods (see ESM 2). Because parental age effects can be confounded by seasonal effects, we included hatching date as a factor. Year was entered as a factor to control for annual effects. To investigate which parental ages were best explaining the variance in nestling traits, we performed a backward model selection on the age of the biological father and mother, and of the foster father and mother by sequentially dropping non-significant terms (*P* > 0.05). RTL was log-transformed throughout analyses. Information on supplementary statistical analyses are reported in ESM 3.

**Results**

RTL of 50-day-old nestlings was significantly related to the age of their biological father and their foster mother after controlling for brood size manipulation, year, and hatching date (Table 1A). Nestlings had shorter telomeres when fathered by older males (Fig. 1A) and reared by older females (Fig. 1B). Effects of the age of the foster father and the biological mother, as well as brood size treatments, year, and hatching date on RTL were non-significant (Table 1A).

Brood size manipulation, but not parental age, negatively affected nestling body mass, wing length, and sternum length, after controlling for year and hatching date (Table 1B-D).

**Discussion**

The individual variation in the age-related patterns of reproductive senescence and survival is a central question in evolutionary biology. Among the implicated factors driving individual differences, parental age at conception has been shown to modulate offspring life-history trajectories [14]. Our experimental cross-fostering design showed that offspring TL at the end of the parental period was negatively related to the age of their biological father and, at a lower level, to that of their foster mother. This suggests that pre-natal paternal effects and post-natal maternal effects, both influenced by parental age, are important determinants of the TL, and that TL could be part of the mechanisms defining the ageing patterns across generations in the long-lived Alpine swift.

Shortening of offspring TL with increasing age of the biological father supports the hypothesis of a prevalence of gametes with shorter telomeres in older swift males. As the telomerase is expected to be active in germ cells throughout their life [15], TL shortening may be induced by a progressive decline in telomerase expression or activity with age, *i.e.* by its incapacity to repair DNA damage that may accumulate more rapidly in old individuals [16]. Through the direct transmission of short telomeres, poor quality sperm of older males may have also impaired the pre- and/or post-natal development of chicks [17], with well-known adverse consequences on telomere length during growth [10]. Interestingly, the age of the biological mother did not affect offspring TL, which does not support the idea that TL erosion in oocytes drives infertility with age in this species, contrary to what has been proposed for humans [8].

While a similar negative effect of father age on offspring TL has been observed in sand lizards [7], such effect was generally not significant in other bird species studied so far. Instead a more prevalent maternal influence has been suggested [18]. This discrepancy may be attributed to the post-natal effects on TL, *i.e.* the environmental conditions experienced during nestling growth. We also found that the age of the foster mother negatively affected TL at fledging. This suggests that female parental care in swifts may help to preserve offspring telomeres during growth, while foster father investment appears of less importance. In Alpine swifts, nestlings depend on their parents for brooding and food provisioning until fledging. A previous study investigating the importance of pre and post-hatching care to chick survival stressed the key role of post-hatching parental care [19]. Since both adults share incubation and feeding duties, the lack of an effect of foster father age on TL may be due to a difference in age-related investment in reproduction of male and female swifts. Remarkably, foster female age was not linked to offspring body mass, size or growth, suggesting that food provisioning was not the prime mechanism influencing TL. This is also supported by the lack of an effect of the brood size manipulation on offspring TL. An alternative explanation could be that poor maternal care influences offspring susceptibility to stress, with potential deleterious consequences for offspring TL erosion [6, 10]. The age of the parents is likely not to be the unique determinant of parental care. Better defining adult individual quality using, for instance, mother and father TL (from biological and foster parents) may lead to a more accurate estimation of the net impact of parental age on offspring TL. Still, determining the TL dynamics of chicks raised by foster mothers, from hatching to fledgling, is required to better define which aspects of early-life parental care influences TL inheritance patterns.

**Ethics statement.** The experiments were approved by the veterinary services of the Canton Berne (no. 51/04) and birds were ringed under the legal authorization of the Swiss Agency for the Environment.

**Data accessibility.** Data available on Dryad Digital Repository. <http://dx.doi.org/10.5061/dryad.ts635> [20]

**Authors' contributions.** P.B. and F.C. conceived the study, PB carried out the field work, S.Z. developed and performed the qPCR measurements. P.B. and F.C. ran the statistical analyses and, with S.Z. for the ESM, drafted the manuscript. All authors gave final approval for publication, declaring no competing interests, and agree to be held accountable for the content therein.

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**Table 1**. Linear mixed-effects models describing the variation in relative telomere length (A), body mass (B), wing length (C), and sternum length (D) of 50-day-old Alpine swift nestlings. The sample size and the partition of the variance are given for each model. The estimate of the effect of brood size manipulation is expressed as reduced vs. enlarged, and that of year as 2004 vs. 2006. Significant effects are reported in bold. The contribution of non-significant effects, when dropped from the model, is reported in italics.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Trait | | Estimate (SE) | | DFDen | t Ratio | Prob>|t| |
|  |  |  |  |  |  |  |
| (A) Relative telomere length | |  |  |  |  |  |
|  | Vorigin = 0.01 ± 0.02 (N = 54 nests), Vrearing = 0.00 ± 0.00 (N = 54 nests), Vresiduals = 0.15 ± 0.03 (N = 95 chicks) | | | | | |
|  |  |  |  |  |  |  |
|  | Brood size manipulation | 0.004 | (0.044) | 71.8 | 0.10 | 0.922 |
|  | Year | -0.037 | (0.060) | 49.5 | -0.61 | 0.546 |
|  | Hatching day | -0.003 | (0.009) | 55.8 | -0.29 | 0.775 |
|  | **Biological father age** | **-0.038** | **(0.017)** | **46.5** | **-2.19** | **0.033** |
|  | **Foster mother age** | **-0.030** | **(0.015)** | **80.6** | **-1.99** | **0.050** |
|  | *Foster father age* | *-0.018* | *(0.022)* | *76.0* | *-0.82* | *0.415* |
|  | *Biological mother age* | *-0.026* | *(0.020)* | *56.0* | *-1.32* | *0.194* |
|  |  |  |  |  |  |  |
| (B) Body mass | |  |  |  |  |  |
|  | Vorigin = 0.00 ± 0.00 (N = 55 nests), Vrearing = 12.57 ± 10.62 (N = 55 nests), Vresiduals = 54.46 ± 11.35 (N = 98 chicks) | | | | | |
|  |  |  |  |  |  |  |
|  | **Brood size manipulation** | **2.895** | **(0.930)** | **53.8** | **3.11** | **0.003** |
|  | **Year** | **2.527** | **(1.173)** | **45.7** | **2.15** | **0.037** |
|  | **Hatching day** | **-0.869** | **(0.174)** | **47.8** | **-4.98** | **<0.001** |
|  | *Biological father age* | *0.019* | *(0.419)* | *38.7* | *0.05* | *0.964* |
|  | *Foster father age* | *0.019* | *(0.453)* | *53.5* | *0.04* | *0.967* |
|  | *Foster mother age* | *0.177* | *(0.343)* | *44.0* | *0.52* | *0.609* |
|  | *Biological mother age* | *-0.207* | *(0.370)* | *89.3* | *-0.56* | *0.577* |
|  |  |  |  |  |  |  |
| (C) Wing length | |  |  |  |  |  |
|  | Vorigin = 0.00 ± 0.00 (N = 55 nests), Vrearing = 29.91 ± 18.88 (N = 55 nests), Vresiduals = 88.62 ± 18.44 (N = 98 chicks) | | | | | |
|  |  |  |  |  |  |  |
|  | **Brood size manipulation** | **4.520** | **(1.259)** | **54.0** | **3.59** | **0.001** |
|  | **Year** | **-4.758** | **(1.595)** | **47.3** | **-2.98** | **0.005** |
|  | **Hatching day** | **-1.541** | **(0.237)** | **49.4** | **-6.50** | **<0.001** |
|  | *Foster mother age* | *0.177* | *(0.490)* | *40.5* | *0.36* | *0.720* |
|  | *Biological mother age* | *0.417* | *(0.512)* | *79.9* | *0.81* | *0.418* |
|  | *Foster father age* | *0.488* | *(0.618)* | *58.1* | *0.79* | *0.433* |
|  | *Biological father age* | *-0.814* | *(0.474)* | *82.7* | *-1.71* | *0.090* |
|  |  |  |  |  |  |  |
| (B) Sternum length | |  |  |  |  |  |
|  | Vorigin = 0.00 ± 0.00 (N = 55 nests), Vrearing = 0.62 ± 0.39 (N = 55 nests), Vresiduals = 1.81 ± 0.38 (N = 98 chicks) | | | | | |
|  |  |  |  |  |  |  |
|  | **Brood size manipulation** | **0.567** | **(0.181)** | **53.2** | **3.14** | **0.003** |
|  | Year | -0.364 | (0.229) | 46.5 | -1.59 | 0.118 |
|  | **Hatching day** | **-0.080** | **(0.034)** | **48.6** | **-2.37** | **0.022** |
|  | *Foster father age* | *0.043* | *(0.093)* | *58.3* | *0.47* | *0.643* |
|  | *Biological father age* | *-0.030* | *(0.070)* | *83.0* | *-0.43* | *0.671* |
|  | *Foster mother age* | *0.091* | *(0.067)* | *45.7* | *1.36* | *0.179* |
|  | *Biological mother age* | *-0.096* | *(0.068)* | *85.6* | *-1.41* | *0.162* |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

**Figure 1**. Relative telomere length in 50-day-old Alpine swift nestlings in relation to the age of their biological father (A) and their foster mother (B). The regression lines (in black) and 95% confidence intervals (in grey) of log-transformed telomere length in relation to parental age are indicated.

