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Fighting for fitness

Salomons, Henri Martijn

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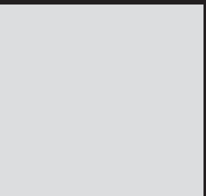

Telomere length variation and sex dependent environmental sensivity during early development in a free-living corvid

H. Martijn Salomons
Ellis Mulder
Simon Verhulst

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Summary

Telomeres, the ends of linear chromosomes, shorten with age, and evidence accumulates that telomere length and shortening rate predicts remaining lifespan. Telomere shortening also reflects life-style and, at least in vitro, is accelerated by oxidative stress. Thus, telomeres may play a crucial role in the mechanism linking environmental conditions, for example those experienced during early development, to individual fitness prospects. However, most studies on telomere shortening to date were correlational and/or on laboratory animals, and consequently little is known of the causes of telomere shortening under natural conditions. We manipulated brood size in free-living jackdaws *Corvus monedula*, and studied effects on telomere shortening in nestlings using pulsed field gel electrophoresis. Telomere shortening in daughters was unaffected by brood size manipulation, despite our earlier finding that their growth was much reduced in enlarged broods. In contrast, telomere shortening in sons in enlarged broods was twice as high when compared to sons reared in reduced broods, while their growth was less affected. We previously showed that oxidative stress was similarly affected by the brood size manipulation (i.e. stronger effects in sons), but we found no correlations between oxidative stress or growth and telomere shortening. This suggests that, in vivo, oxidative stress does perhaps not play an important role in telomere dynamics in nestlings. Our findings do contribute to the view that the shortening of telomeres may serve as a valuable indicator of environmental conditions during development. However, further study is required, both to elucidate the mechanism causing the brood size effect on telomere shortening, and its fitness consequences.

Introduction

Environmental conditions experienced during early development have effects on the fitness prospects in animals (Lindström 1999; Cam et al. 2003; van de Pol et al. 2006) and humans (Lummaa 2003). For instance, poor environmental conditions experienced during early development affect survival rates and lead to costs in terms of immunity and/or reproductive potential through decreased fertility or attractiveness (Haywood & Perrins 1992; Gustafsson et al. 1995; de Kogel & Prijs 1996; Metcalfe & Monaghan 2001; Fargallo et al. 2002; Lummaa & Clutton-Brock 2002) as well as metabolic rate at adulthood, independent of body size (Verhulst et al. 2006; Criscuolo et al. 2008). Considering our knowledge on linking early environmental conditions to individual fitness prospects, surprisingly little is known on the mechanisms underlying these effects. Especially interesting in this context is evidence showing that the rate of growth can be more important than the amount of growth (Rollo 2002). Apparently, physical speed of growth might be attained at the expense of the developing physiological machinery of organisms. This becomes especially visible in the case of 'catch-up' or compensatory growth, an acceleration of growth rate caused by an increase in nutritional value of food provisioning during development, often leading to increased adult mortality risk, metabolic rate and disease (Metcalfe & Monaghan 2001; Metcalfe & Monaghan 2003; Barker et al. 2005; Criscuolo et al. 2008).

Telomeres are regions of non-coding but highly structured DNA at the end of linear eukaryotic chromosomes, consisting of a tandem repeated DNA sequence (5'-TTAGGG-3')n, a sequence that is highly conserved. They play an important role in the protection of chromosome integrity (Blackburn 1991), and telomere shortening is often associated with ageing and age-related diseases (e.g. cardiac and infectious diseases, artherosclerosis and cancer) (Cawthon et al. 2003; Baird 2006; Jiang et al. 2007). There are indications that telomere length predicts remaining life span in humans (Cawthon et al. 2003; Bakaysa et al. 2007; Kimura et al. 2008) (but see (Aviv 2008)), birds (Haussmann et al. 2005; Salomons et al. 2009b) and nematodes (Joeng et al. 2004). Moreover, when comparing species, telomere shortening rate is correlated with maximum lifespan, in that short lived species loose their telomeres at a higher rate (Haussmann et al. 2003), but note that between species the average telomere length is not correlated with (maximum) life span (Haussmann et al. 2003; Seluanov et al. 2007). Telomere length is at least partly genetically determined (Slagboom et al. 1994; Rufer et al. 1999; Graakjaer et al. 2006), there is however large variation in the rate of telomere shortening between individuals (Salomons et al. 2009b).

There have been several studies looking at age related changes in telomere length providing estimates of telomere shortening of humans and other vertebrates early in life (Frenck 1998; Rufer et al. 1999; Zeichner et al. 1999; Brummendorf et al. 2002; Hall et al. 2004; Salomons et al. 2009b). Generally, telomere shortening early in life is markedly higher compared to that in adults. In jackdaws, the rate at which telomeres shortened between ages 5 and 30 days was up to 100 times higher than the rate recorded in adults (Salomons et al. 2009b). Obviously, telomere length during adult life is predominantly affected by the amount of telomere loss in early life. Furthermore, evidence from the European shag (*Phalacrocorax aristotelis*) indicated that telomere shortening was linked to growth and to relative time in the breeding season (Hall et al. 2004), suggesting that environmental conditions during early development are related to telomere shortening rate. We are aware of only one previous experimental study investigating this relationship. Jennings and colleagues showed that changes in early growth induced by diet restriction affected telomere shortening (Jennings et al. 1999).

Telomere shortening rate has been shown, at least in vitro, to be accelerated by oxidative stress (von Zglinicki 2002; Tchirkov & Lansdorp 2003), resulting from the imbalance between pro-oxidant production and the capability of an individual to defend itself against and/or repair damage caused by these pro-oxidants (Ames et al. 1993; Beckman & Ames 1998; von Schantz et al. 1999; Finkel & Holbrook 2000; Kregel & Zhang 2007; Costantini 2008). Anti-oxidant protection may be costly, as suggested by its suppression in birds with increased reproductive effort (Wiersma et al. 2004). Moreover, the level of anti-oxidant protection in nestlings is primarily determined by the rearing environment (e.g. food delivery by the parents) (Blount et al. 2003; Costantini & Dell'Omo 2006; Rubolini et al. 2006; Isaksson et al. 2007). Therefore, the damage incurred by oxidative stress (either through or approximated by telomere length) may provide a link between environmental conditions experienced during early development and individual fitness prospects.

We manipulated brood size in free-living jackdaws and measured telomere length at two time points in the nestling phase. Life history theory predicts a trade-off between the number and quality of offspring which has been confirmed by many studies (Smith et al. 1989; Stearns 1992; Pettifor et al. 2001). Thus, increasing or decreasing the number of young allowed us to compare the effects of either impaired or improved environmental conditions during the rearing period of nestlings on telomere length at fledging. Previously we have shown that sub-optimal conditions in ‘enlarged’ broods resulted in lower growth (body mass and tarsus length) and especially in altered levels of plasma anti-oxidative barrier (TAC) and oxidative damage in nestlings reared under these conditions. Interestingly, these effects were different between daughters and sons. Growth was most strongly affected in daughters. In contrast, oxidative stress related parameters were more affected in sons. Although levels of TAC were, as expected, lower in the plasma of nestlings reared in ‘enlarged’ broods, oxidative damage levels were higher in sons reared in ‘reduced’ broods. We hypothesized that the different responses of daughters and sons to brood size manipulation were the result of differential allocation of resources. Whereas sons, possibly as a result of selective pressures on body size, invested in growth at the expense of physiological consequences under sub-optimal conditions, daughters allocated more resources towards maintenance and repair. This is the first study in which the combined effects of early environment on growth, oxidative stress as well as telomere shortening are studied within (free-living) individuals. We expected telomere shortening of nestlings reared in ‘enlarged’ broods to be higher (independent of growth) because the sub-optimal rearing conditions (based on the effects on growth) for nestlings in ‘enlarged’ broods would result in a lower availability of resources for somatic maintenance and repair. We further predicted that this effect would be more pronounced in sons because the oxidative stress assays already showed their physiology to be stronger affected than in daughters.

Methods

Study population

We studied free-living jackdaws, a hole breeding semi-colonial bird species, in the colony at the Biological Centre (BC) in Haren (The Netherlands), a semi-urban environment, and 5 smaller colonies located in a more rural area 5–10 kilometres south of Haren. The BC colony consisted of 36 nest boxes. At the other sites, the first nest boxes were put up in 2004 and depending on jackdaw occupation rates their number was increased in consecutive years. The number of nest boxes per site ranged from 5–20. Due to relative low occupation rates at some of these sites, data were pooled for the colonies outside the Biological Centre (OTHER).

Nest boxes were checked daily, starting in the first week of April, until the clutch was complete, and eggs were numbered with a felt tip pen. For the BC colony, clutches were moved to an incubator 1–2 days before the estimated hatching date (temperature 37.7ºC, humidity 75%), and upon hatching, hatchlings were placed in their original nest (for details see Salomons et al. 2006). At the other colonies nests were checked daily for hatched eggs, starting one day before estimated hatching date. Hatchlings were weighed and a blood sample (10–20 µl) was taken by clipping the tip of a toenail for sexing and future DNA-analysis. Sex was determined by PCR analysis of blood samples (Griffiths et al. 1998). The reliability of this method was confirmed using adult birds of known sex (*N* > 50). The clipping of a nail tip does not interfere with nestling growth. The clipped nail is identifiable by a blunt tip up to fledging, and we used this to identify the chicks within broods.

Brood size manipulation

We manipulated brood size at day 5 or 6 (day of hatching = day 1). Nests with only one chick alive in the nest at the day of manipulation were not used, as these nests could only be enlarged. Parents were randomly assigned to an ‘enlarged’ or ‘reduced’ treatment in their first ‘manipulation year’. Parents stayed on this treatment in consecutive years, to study long-term effects of increased reproductive effort. For ‘reduced’ broods, two chicks were moved to same aged broods in the ‘enlarged’ treatment. In 2005 and 2006 a full cross-foster scheme was applied where three chicks were moved from the ‘reduced’ brood to the ‘enlarged' brood and one chick from the ‘enlarged’ brood was moved to the ‘reduced’ brood.

Growth and survival

The survival of the chicks in the nest was checked every 5 days (day of hatching = day 1). At day 10, 20 and 30 the chicks were also weighed and tarsus- and wing length (day 20 and day 30) were measured. As body mass is a combined measure of size and condition, we separated these components in our analysis by replacing body mass in the model by tarsus and residuals of the regression of mass over tarsus (including sex * tarsus interaction). At day 30, shortly before fledging, the chicks were ringed.

Telomere length analysis

At the age of 5 days, at the same time as the brood size manipulations, we again clipped the nail tip of one of the toes of each chick to create combinations of clipped nail that were unique within the rearing nest after the brood size manipulation. From the clipped nail a small blood sample (~40 µl) was taken for DNA. At the age of 30 days all remaining nestlings were again sampled, this time by puncture of the brachial vein. Both samples were stored in 2% EDTA buffer at a temperature of 4–7 °C, before it was snap-frozen, within two weeks after sampling, in a 40% glycerol solution for permanent storage at –80°C. All samples were collected under license from the Animal Experiments Committee of the University of Groningen.

We used erythrocytes as source of DNA, which was extracted from the nuclei of ± 5 µl isolated erythrocyte cells using CHEF Genomic DNA Plug kit (Biorad, Hercules, CA). Cells in the agarose plug were digested overnight with Proteinase K at 50°C. The genomic DNA within half of this plug was digested simultaneously with Hind III (60 U), Hinf I (30 U) and Msp I (60 U) for ~18 h in NEB2 buffer (New England Biolabs, Inc, Beverly, MA). Approximately 5 µg of digested DNA from each sample was separated by pulsed field gel electrophoresis through a 0.8% pulsed field certified agarose gel (Bio-Rad, Hercules, CA) at 14°C for 24 h (3 V/cm, initial switch time 0.5s, final switch time 7.0s). Gels were dried using a gel dryer (Bio-Rad, model 538) without heating and hybridized overnight with a ^{32}P-endlabeled oligo (5′-CCCTAA-3″)$_4$ that binds to the 3″ overhang of telomeres, thereby avoiding the problem of interstitial telomeres that are preva-

lent in birds (Class I telomeres (See Delany et al. 2000 for a description of different telomere classes)). The radio-active signal of the marker was detected by a phosphor screen (MS, Perkin-Elmer) and analysed using a phosphor imager (Cyclone™ Storage Phosphor System, Perkin-Elmer). For size calibration we used a ^{32}P labeled size ladder (NEB DNA ladder 1 kb).

We determined the size distribution of telomeres through densitometry using the open-source software *ImageJ version 1.38x*. See Salomons et al (submitted) for further details. TRF length varies between chromosomes (Baird et al. 2003), and due to stochastic events also between the hematopoietic stem cells that generate the erythrocytes, and hence the data obtained for each sample is a TRF distribution (a smear) rather than one value (a sharp band). The TRF distribution is usually characterized using the average TRF only, but, as explained above, TRF shortening rate may not be the same for chromosomes with short and long TRF's. We therefore characterized the TRF distributions not only by the mean, but also by the percentiles, and examine TRF shortening separately for these different estimates.

Statistical analysis

We manipulated brood size of 90 nests, containing in total 325 nestlings ($N = 14$, 24, 17, 35 broods for 2005-2008 respectively). At manipulation, there were no significant differences between 'enlarged' and 'reduced' broods in either laying date, hatching date, clutch size, brood size, nestling mass or telomere length (all $P > 0.1$). For 76 nests two chicks were either removed from or added to the original brood. In one occasion three chicks were moved from 'reduced' to 'enlarged'. In 12 nests only one chick could be moved because there were only two (viable) chicks left in the nests assigned to the 'reduced' treatment at the time of the manipulation. Since there was an association between original brood size and the number of chicks manipulated, we treated the manipulation as a categorical variable with two levels.

Averaging over years and colonies, there were on average 2.1 ±0.1 and 5.3 ±0.2 chicks in reduced and enlarged broods respectively after manipulation. Estimates of telomere length at day 5 were available for 229 nestlings (2005: $N = 45$; 2007: $N = 58$; 2008: $N = 126$), for technical reasons there were no samples for 2006), and for 163 nestlings telomere length was determined at day 30 (2005: $N = 35$; 2007: $N = 47$; 2008: $N = 81$). For a total of 136 nestlings telomere length was determined both at day 5 and day 30 (2005: $N = 28$; 2007: $N = 42$; 2008: $N = 66$)). In total, 183 nestlings for which telomere length at the age of five days was determined, were reared in nests where brood size was either enlarged ($N = 129$) or reduced ($N = 54$). For 108 of these nestlings a second measure of telomere length was determined ('reduced': $N = 75$; 'enlarged': $N = 33$).

In total we measured TRF length of 385 samples on 18 gels. As we were mainly interested in within individual changes in telomere length, samples of those individuals for which two samples were available were run on the same gel, to minimise measurement error. We analyzed our data using mixed models, incorporating random effects to avoid pseudo-replication. We used JMP (version 7.0.1, SAS Institute Inc.) for all analyses except for the survival of nestlings where we used MLWiN (version 2.0.2, Rasbash et al. 2005) to build a logistic model with random terms. Siblings are not statistically independent; therefore biological mother and nest were added as random factors to all models.

Results

Initial telomere length

Approximately half of the variation in telomere length at day 5 was explained by parental effects (mother: $r = 0.54 \pm 0.07$ $F_{58,169} = 5.5$ $P < 0.001$, father: $r = 0.49 \pm 0.07$ $F_{57,170} = 4.8$ $P < 0.001$; estimates calculated following Lessells and Boag (1987), s.e. calculated following Becker (1984)). This is in line with data showing that

offspring telomere length is at least partly heritable in humans (Slagboom et al. 1994; Rufer et al. 1999; Graakjaer et al. 2006). Repeatability calculated for females ($N = 9$) of which offspring telomere length was measured in two or three separate years was approximately the same ($r = 0.40 \pm 0.3$ $F_{7,8} = 2.3$ $P = 0.1$), for males this value was however considerably lower and non-significant ($r = 0.12 \pm 0.3$ $F_{10,11} = 1.3$ $P = 0.4$). This indicates that it was indeed a 'parental effect' rather than a 'nest-effect' and that this effect was primarily determined via the mother. None of the parameters that characterise individual nests (laying date, clutch size, brood size, sex ratio of brood mates, colony, body condition of the parents or their interactions), nor parameters that characterise individual nestlings (body mass, sex, exact age) were correlated with telomere length at day 5 (Table 4.1).

Telomere shortening

On average, nestlings telomeres shortened by 270.0 ±19.7 bp between day 5 and day 30 (Figure 4.1; $t_{35.4,92.5} = 13.8$, $P < 0.001$). Although

Table 4.1. Analysis of the effect of several parameters on telomere length early in the rearing period.

Parameter of interest	Estimate (Standard Error) [1]	*DFDen*	*t* Ratio	*P*
Clutch size (*N*=229)		34.3	0.7	0.7
CS=3	-39.2 (156.6)			
CS=4	-60.2 (113.6)			
CS=5	8.2 (103.7)			
CS=6	-212.2 (160.8)			
Average egg volume (*N*=223)	-69.2 (62.3)	61.3	-1.1	0.3
Hatching date (*N*=229)	11.6 (12.4)	65.1	0.9	0.4
Brood size at day 5 (*N*=229)		8.3	0.8	0.7
NC5=2	93.7 (147.4)			
NC5=3	-15.9 (147.0)			
NC5=4	-113.8 (127.5)			
NC5=5	-142.5 (138.0)			
NC5=6	5.7 (261.4)			
Sex ratio (*N*=218)	-21.6 (74.0)	198.1	-0.3	0.8
Maternal condition (*N*=93)	-13.9 (14.3)	1.0	-1.0	0.5
Paternal condition (*N*=93)	-2.2 (7.7)	33.9	-0.3	0.8
Egg volume (*N*=96)	18.6 (43.8)	76.7	0.4	0.7
Hatching order (*N*=97)		62.6	0.9	0.5
Order=2	-14.6 (62.0)			
Order=3	109.7 (70.6)			
Order=4	60.0 (77.7)			
Order=5	-159.6 (118.3)			
Sex (Son) (*N*=219)	35.0 (51.0)	175.6	0.7	0.5
Body mass (*N*=219)	-2.8 (2.2)	194.0	-1.3	0.2
Age (*N*=229)		152.9	0.8	0.7
Age=3	70.6 (77.8)			
Age=4	31.4 (71.3)			
Age=5	-74.4 (93.6)			
Age=6	-143.8 (211.0)			

[1] Estimates of categorical variables represent deviation from reference category (between brackets)

rate of telomere shortening was higher in daughters compared to sons (302.5 ±27.2 vs 242.4 ±26.0) this difference was not significant (N = 108, $t_{1,100.2}$ = –1.6 P = 0.1).

The effect of the brood size manipulation on telomere shortening was much stronger in sons than in daughters, indicated by a significant manipulation*sex interaction (Figure 4.2; $t_{1,102.9}$ = –2.0 P < 0.05). Sons in enlarged broods lost almost twice as many base pairs compared to sons in reduced broods (148.9 ±49.2 vs. 292.2 ±35.1; $t_{1,52.2}$ = –2.2 P = 0.03), while in daughters the difference was negligible (326.7 ±46.5 vs 294.7 ±30.0; $t_{1,47.7}$ = 0.7 P = 0.5). When the sexes were pooled, telomere shortening was still higher in nestlings in 'enlarged' broods (Figure 2), but this effect was not statistically significant ($t_{1,105}$ = –1.6 P = 0.1).

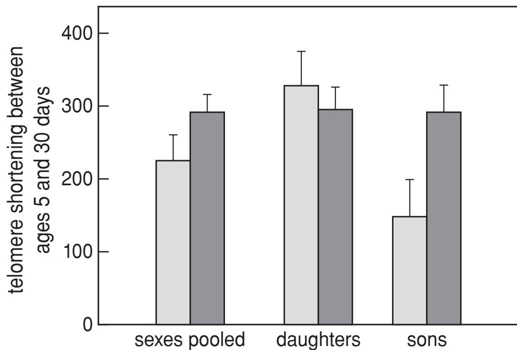

Figure 4.1. Telomere shortening for nestlings reared in broods where the number of nestlings was reduced (light grey bars) or enlarged (dark grey bars). Effects are shown for a data set with both sexes pooled as well as for daughters and sons separately.

Using percentiles to characterize the size distribution of telomeres within separate samples we tested whether the effect of brood size manipulation on telomere shortening differed for telomeres of different sizes (see Figure 4.4). The effect was fairly constant over the entire size distribution, indicating that the increase in telomere shortening in nestlings reared in 'enlarged' broods was not proportional to the length of individual telomeres but similar for all telomeres within an individual.

Telomere shortening and oxidative stress

The effect of brood size manipulation on telomere shortening was very similar to the previously observed effect on levels of oxidative

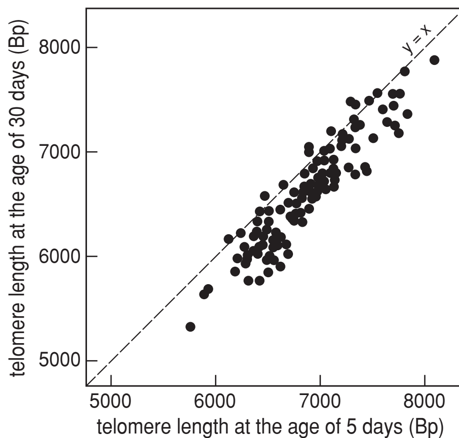

Figure 4.2. Nestling telomere length at ages 5 and 30 days plotted against each other. Line indicates equal values (y = x) and hence when telomeres have shortened between ages 5 and 30 days the data points fall below this line.

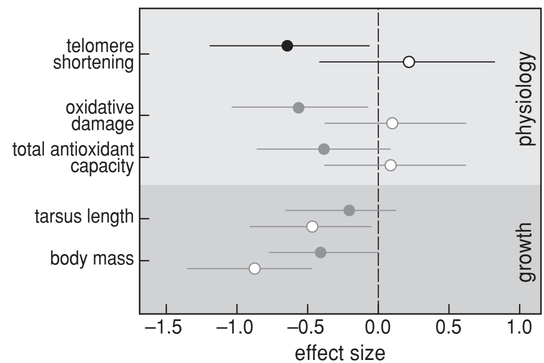

Figure 4.3. Comparison of the effect size of the brood size manipulation for different parameters in daughters (open dots) and sons (closed dots). Effect sizes in grey are from (Salomons et al. 2009a). Dashed line indicates no effect.

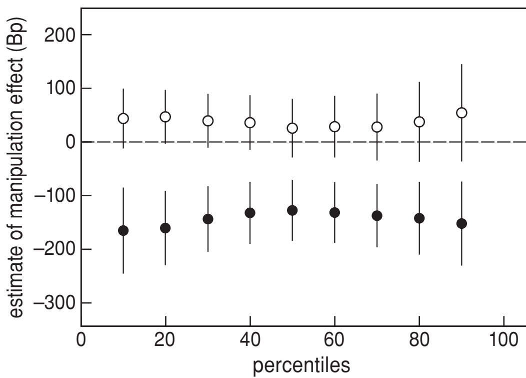

Figure 4.4. Effect of brood size manipulation on telomere length at fledging for different parts of the size distribution in daughters (open dots) and sons (closed dots). Dashed line indicates no effect.

stress (see Figure 4.3). As oxidative stress is in vitro often linked to telomere shortening, our data seem to support this relation also in vivo. However, we found no significant correlations between either growth or levels of oxidative stress with telomere shortening in our nestlings. There was a trend that telomere shortening was higher in nestlings with either low or high levels of plasma anti-oxidant barrier (see Table 4.2). This effect of anti-oxidant protection was far from significant when the sexes were tested separate, and also did not affect the observed relation between brood size manipulation and telomere shortening.

Table 4.2. Analysis of the effect of several parameters on telomere shortening

Parameter of interest Telomere shortening (+ = more shortening)	Estimate (Standard Error) [1]	*DFDen*	*t* Ratio	*P*
Manipulation (enlarged)	29.4 (59.5)			
Sex (son)	168.4 (66.2)			
Sex * Manipulation	-163.0 (80.3)	102.9	-2.0	0.04
Hatching date (*N*=108)	-2.2 (6.8)	38.6	-0.3	0.7
Average egg volume (*N*=102)	32.2 (28.9)	38.0	1.1	0.3
Maternal condition (*N*=95)	-1.5 (2.2)	21.0	-0.7	0.5
Paternal condition (*N*=93)	-2.2 (2.1)	31.8	-1.1	0.3
Sex ratio (*N*=100)	119.3 (62.0)	53.2	1.9	0.06
Colony (BC) (*N*=108)	-0.6 (23.1)	28.5	-0.03	1.0
Hatching order (*N*=40)		22.9	1.1	0.3
Order=2	-112.0 (64.5)			
Order=3	18.7 (87.3)			
Order=4	122.5 (98.8)			
Order=5	-38.7 (114.9)			
Age at day 5 (*N*=108)		64.3	1.8	0.1
Age=3	-79.1 (51.7)			
Age=4	-28.4 (35.7)			
Age=5	64.0 (43.4)			
Age=6	93.5 (78.9)			
Body mass (*N*=107)	-0.2 (0.7)	62.2	-0.3	0.8
Tarsus length (*N*=108)	-5.8 (13.5)	94.1	-0.4	0.7
Anti-oxidants	0.9 (1.7)	77.1	0.5	0.6
Anti-oxidants^2	-0.2 (0.1)	77.5	-1.9	0.06
Oxidative damage	-1.2 (2.4)	76.7	-0.5	0.6

[1] Estimates of categorical variables represent deviation from reference category (between brackets)

Discussion

Experimentally manipulated environmental conditions during early development indeed affected telomere shortening in the offspring. This effect of the brood size manipulation on telomere length depended on the sex of the offspring: telomere shortening was higher in sons reared in 'enlarged' broods compared to sons reared in 'reduced' nests, whereas levels of telomere shortening were not different between treatments in daughters (Figure 4.1). We further showed that while both shortening rate and the predictive value for mortality are higher for longer individual telomeres (Salomons et al. 2009b), the manipulation effect was fairly constant over the different percentiles (see Figure 4.4). This indicates that the increase in the amount of attained damage between treatments was not proportional to the length of individual telomeres. As telomere length and especially telomere shortening rate was previously shown to be correlated to survival in the jackdaw (Salomons et al. 2009b), the increase in telomere shortening rate in nestlings reared in enlarged broods observed in our data could very well provide a link between early environmental conditions and fitness.

Oxidative stress accelerates telomere shortening in vitro (von Zglinicki 2002; Tchirkov & Lansdorp 2003). However, evidence for a direct link between oxidative stress and telomere shortening has thus far not been shown in vivo. Our data do suggest that higher levels of TAC in sons reared in 'reduced' broods resulted in a better defense against oxidative damage. However, the relation between telomere shortening and levels of TAC did not quite reach significance. TAC also did not change the relation between brood size manipulation and telomere shortening, ruling out TAC as the mediator of this relationship. Moreover, besides the higher level of anti-oxidative protection in sons reared in 'reduced' broods, surprisingly also the level of oxidative damage was higher in these nestlings. And finally, there was no direct correlation between oxidative damage and telomere shortening. As previously suggested by others (Barja 2002; Costantini 2008), levels of oxidative protection can be up regulated to compensate for increased levels of pro-oxidants. This could explain the fact that we found telomere shortening to be highest in sons in 'enlarged' broods while levels of oxidative damage were highest in sons reared in 'reduced broods'. Even though we found no significant interaction effects between oxidative damage and anti-oxidant barrier on telomere shortening, the aforementioned compensatory mechanisms might explain the absence of a direct link between telomere shortening and either oxidative damage or anti-oxidant barrier separately in this study. There is of course the possibility that we failed to find a relation because of practical reasons, for instance it could be that there was a mismatch in the timing of our sampling, as we took only one sample to estimate levels of oxidative stress during the nestling phase. Therefore we want to stress here that we do not see the fact that we found no relation between levels of oxidative stress and telomere shortening as proof that such a relation does not exist in vivo.

Sex dependent effects of environment during early development have been reported more often (Martins 2004; Råberg et al. 2005; Dubiec et al. 2006; Arnold et al. 2007; Alonso-Alvarez et al. 2007; Rowland et al. 2007). For instance, Martins (Martins 2004) showed that in hand reared zebra finches, in the absence of parent-offspring and sib-sib interactions, final body mass and growth rates of females were lower in conditions of restricted food when compared to males subjected to the same restriction protocol. This is interesting because not only could this affect population sex ratio responses to environmental variation, it may also create selective pressure to invest in a specific sex in response to state, condition and/or environment of the parents, thereby facilitating the evolution of adaptive sex allocation. However which sex is most susceptible to the

manipulation varies between species, but also between studies on the same species (Råberg et al. 2005). Previously, we have shown that, at first glance, daughters suffered most when brood size was enlarged in terms of growth. Here we provide further proof that sons also paid, less visible, costs in terms of physiology. This further supports the hypothesis that the observed effects were the result of differential energy allocation in the trade-off between growth and somatic maintenance and that especially sons would, under sub-optimal conditions, invest most in growth at the expense of physiology as a result of selective pressures. Such selective pressures could arise for example through an effect of body size on social dominance, as in jackdaws, dominance rank has been shown to affect future survival and reproductive success (Henderson & Hart 1995; Verhulst & Salomons 2004) and the rank of a breeding pair is primarily dependent on the status of the male (Röell 1978). Hence, by investing in growth under sub-optimal conditions at the expense of other physiological parameters, jackdaw sons may increase their fitness. For daughters on the other hand, body size may be less important and more resources are allocated towards physiological maintenance and repair. Our data on telomere shortening support this hypothesis in that sons reared under sub-optimal conditions indeed pay physiological costs, while telomere shortening was independent of brood size manipulation in daughters.

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