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Telomere dynamics from hatching to sexual maturity and maternal effects in the “multivariate egg”

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ABSTRACT

Avian eggs contain a large number of molecules deposited by the mother that provide the embryo with energy but also potentially influence its development *via* the effects of maternally-derived hormones and antibodies: the avian egg is “multivariate”. Multivariate effects on offspring phenotype were evaluated in a sister study on captive zebra finches, by simultaneously manipulating maternally derived antibodies after LPS-treatment of mothers and egg-treatment using yolk testosterone injection. LPS-treatment had a positive effect on body mass growth at 30 days after hatching and immune response at sexual maturity, while egg-testosterone positively influenced immune response at fledging and courtship behaviour in sexually mature male offspring. Maternal effects are known to modulate offspring telomere length. Still, the multivariate effects of egg-derived maternal components on offspring telomere dynamics from hatching to sexual maturity are undefined. Here, using the data of the sister study completed with telomere measurements, we tested a) the effects of LPS and T treatments on telomere length (TL) from hatching to sexual maturity (day 82), b) how LPS treatment modulated telomere length over reproduction in adult females, and c) the relationship between maternal and offspring TL. We predicted, a) TL would be shorter in LPS fledglings (as a cost of faster growth), and b) TL would be longer in sexually mature adults from T-treated (as a proxy of individual quality). In adult females, there was an overall negative relationship between laying and rearing investments and telomere length, this relationship being weaker in LPS treated females. In chicks, there was an overall negative effect of LPS treatment on telomere length measured at fledging and sexual maturity (day 25 – 82). In addition, at fledging, there was a sex x LPS x T-treatment interaction, suggesting the existence of antagonistic effects of our treatments. Our data partially support the hypothesis of telomeres are proxies of individual quality and that individual differences in telomere length are set-up very early in life.

45

46 **INTRODUCTION**

47 Telomeres are non-coding DNA sequences that control DNA-end recognition and fusion in
48 linear chromosomes (Blackburn, 1991). However, because of the non-replication of the 3'-end of
49 DNA during cell division, telomeres shorten over the lifetime of a cell (Blackburn, 2000). In addition,
50 telomere ends seem to be preferred targets of oxidative stress, an observation albeit currently better
51 documented at the cellular (von Zglinicki, 2002) than at the whole organism level (Boonekamp et al.,
52 2017; Reichert and Stier, 2017). Telomere maintenance mechanisms also exist, which may counter-
53 balanced telomere erosion (Beery et al., 2012; Blackburn and Epel, 2012) and studies across a range
54 of taxa have shown that telomere dynamics are more complex than a simple, regular rate of
55 shortening over age (Fairlie et al., 2016). In fact, several recent studies have highlighted evidence for
56 telomere lengthening in different ecological or experimental conditions (Hoelzl et al., 2016a; Hoelzl
57 et al., 2016b; Lieshout et al., 2019; Spurgin et al., 2018). This idea of negative/positive telomere
58 dynamics has led to the suggestion that telomeres represent one of the cell mechanisms
59 underpinning life-history trade-offs, and that they are a key molecular tool to explore inter-individual
60 differences in ageing trajectories and fitness (Young, 2018). The growth – ageing trade-off and its
61 concomitant changes in telomere length have attracted extended interest (reviewed in (Monaghan
62 and Ozanne, 2018)) because damaged or shortened telomeres are good candidates explaining future
63 consequences of variable offspring investment in the soma (Monaghan and Haussmann, 2006;
64 Reichert et al., 2015a). Indeed, telomere erosion during development has the potential to effectively
65 mediate the effect of early-life stress, being predictive of future individual fitness (Heidinger et al.,
66 2012; Marasco et al., 2019). Accordingly, telomere length at the end of growth correlates with
67 remaining lifespan in birds and mammals (*e.g.* (Heidinger et al., 2012; Lieshout et al., 2019)), even
68 though causality between telomere length and fitness traits remains an open question (Simons,
69 2015).

70 Growth is a period of extensive cell division and of high energy expenditure (2-6 times basal
71 metabolic rate, (Dunn, 1980; Kirkwood, 1991; Vleck and Vleck, 1980)), both of which potentially
72 influence telomere erosion in early-life (albeit putatively at different degrees, see (Boonekamp et al.,
73 2017)). As such, fast growth is predicted to be costly in terms of telomere length (Jennings et al.,
74 1999; Metcalfe and Monaghan, 2001). Numerous previous studies conducted on birds have
75 addressed how growth trajectories and telomere dynamics are intertwined but have produced
76 equivocal results (reviewed in (Vedder et al., 2017)). Most studies report negative effects of stressful
77 growth environments on chick telomeres but rarely confirm a causal relationship with (a high)
78 growth rate (*e.g.* (Boonekamp et al., 2014; Reichert et al., 2015a; Voillemot et al., 2012)). Growth
79 rate has evolved as a compromise between the benefit and the costs of rapid growth for new born
80 organisms in any given environment (Dmitriew, 2011). The environment in which early-life growth
81 takes place is thus widely recognized to affect the phenotype through ontogeny. In this early-life
82 environment, the non-genetic effects derived from the mother's physiological or behavioural traits,
83 so-called maternal effects, are of prime importance in determining the offspring phenotype
84 (Mousseau and Fox, 1998). For instance, pre-natal adjustments in yolk components like hormones
85 should have additive effects on the genetic program of development of the embryo (Groothuis et al.,
86 2005). Thus, maternal effects may drive the adaptation of offspring phenotype to current
87 environmental conditions (*i.e.* maternally-derived adaptive phenotypic plasticity), including the
88 modulation of trade-offs with body maintenance and their ageing consequences. A large panel of egg
89 components, including macronutrients, hormones and antibodies, might act as developmental
90 regulators. This diversity of egg components that can putatively modulate the phenotype of the
91 developing organism (in addition to their interactions) has led to the concept of the "multivariate
92 egg" (Postma et al., 2014; Williams and Groothuis, 2015). Because virtually all components of the egg
93 may have their own effects, and potentially act in a synergistic or antagonistic ways, this requires
94 integration of multiple interactive factors when studying maternal effects on offspring phenotype
95 (Possenti et al., 2018; Torres et al., 2019). For example, manipulation of yolk-deposited carotenoids

96 and testosterone showed that their independent deleterious effects on chick oxidative stress, while
97 triggering a higher growth rate, were buffered when both factors were increased simultaneously
98 (Giraudeau et al., 2017). Interestingly, deleterious effects of steroid hormones on telomere length
99 have been previously shown (e.g. testosterone, (Drury et al., 2014)), providing a potential
100 mechanism by which maternal effects may modulate telomere shortening in offspring (Marchetto et
101 al., 2016). In birds, pre-natal as well as post-natal maternal investment in reproduction have been
102 studied in the context of telomere length of avian chicks: telomere length decreases with laying
103 order (Noguera et al., 2016) and with increased egg content in corticosterone (Hausmann et al.,
104 2012; Tissier et al., 2014); instability in incubation temperature shortened telomere length at
105 hatching (Stier et al., 2019) while lower incubation temperature decreased growth rate and telomere
106 erosion (Vedder et al., 2018). In addition, telomere shortening is associated with immune system
107 activation in chicks and adult birds (Asghar et al., 2015; Lardy et al., 2018). In this case, telomere
108 erosion may be due to higher rate of cell division or to side deleterious effects of the production of
109 oxidative molecules by immune cells (Criscuolo et al., 2018). Using lipopolysaccharide (LPS) injection
110 in mothers before egg-laying (Boulinier and Staszewski, 2008; Torres et al., 2019), the impact of yolk-
111 deposited maternal antibodies on offspring telomeres can be experimentally tested.

112 Maternal effects on telomere length are part of the broader question of how telomere
113 length is passed on to the next generation, *i.e.* how gene x environment interactions determine
114 telomere inheritance (Dugdale and Richardson, 2018). Early studies suggested telomere length to be
115 mainly genetically determined (Graakjaer et al., 2004) and with a more pronounced mother-offspring
116 resemblance in birds ((Becker et al., 2015) but see (Atema et al., 2015)), but contrasting data are
117 accumulating suggesting a key influence of early-life environmental conditions in parents / offspring
118 telomere resemblance, like parental care-derived effects (Viblanco et al., 2020). Among those a role
119 for epigenetic-like effects from paternal gametes have been recently underlined in birds (Bauch et
120 al., 2019), but the potential role of maternally-derived egg effects on offspring telomeres remains
121 relatively unknown.

122 Until recently, most studies on telomere dynamics have focused on effects of early life
123 conditions and development rather than changes in early adulthood. This is likely to have important
124 consequences for our understanding of how adult telomere dynamics might play a role in trade-offs
125 between current vs. future reproduction or adult survival (Bauch et al., 2013; Bichet et al., 2020;
126 Reichert et al., 2014). This is based on the hypothesis that telomere maintenance is energy-
127 demanding and thus telomere length will reflect trade-offs (Bauch et al., 2016; Young, 2018).
128 However, another possibility is that individual differences are mainly established during
129 development, at birth or very early in adulthood (*i.e.* before adult maturity), which may indicate that
130 individual variation in telomere length is only weakly affected by environmental stress or trade-offs
131 over adult life (Bichet et al., 2020). As telomere length has been related to adult foraging efficiency
132 (Young et al., 2015) or breeding success (Bauch et al., 2014), it was suggested to be a biomarker of
133 individual quality (Angelier et al., 2019; Le Vaillant et al., 2015), individual quality being mostly
134 interpreted as a multivariate characteristic inherited from parents and then further modulated very
135 early in life.

136 The present study follows up on the study by Torres et al. (Torres et al., 2019), which focused on
137 multivariate maternal effects on offspring phenotype. This previous study showed that LPS
138 treatment did not change the reproductive output of females, and had only a small effect on growth
139 trajectories of chicks: male offspring of LPS challenged females were heavier when 30 days-old
140 (Torres et al., 2019). However, the immune response of chicks to a phytohemagglutinin test (PHA)
141 was higher in chicks from T-treated eggs at day 26 after hatching (end of growth), and in chicks raised
142 by LPS-treated mothers at day 82 after hatching (sexual maturity). In addition, courtship behaviour of
143 males was significantly and positively affected by the testosterone treatment, although there were
144 no interaction effects of LPS and testosterone on the chick phenotype. Thus, male chicks could have
145 benefited from (i) mother's LPS challenge (*i.e.* being larger at day 30) and (ii) egg-testosterone
146 injections, being of higher quality than control males at sexual maturity. Similarly, testosterone and
147 LPS chicks had a better immune response at the end of growth and at sexual maturity. In the present

148 study, we considered telomere dynamics in adult female zebra finches (*Taeniopygia guttata*) during
149 reproduction and in their chicks (offspring) from hatching to sexual maturity. Our predictions rely on
150 the adult and immature phenotypes previously observed (Table 1). We present data on: a) changes in
151 telomere length in adult female zebra finches in relation to reproductive investment, including
152 females that were LPS immune-challenged prior to egg production: we predicted that telomeres of
153 mothers should shorten in relation to reproductive effort (*i.e.* with increasing clutch/brood size) and
154 this effect would be enhanced in immune challenged females; b) the relationship of telomere length
155 between parents (mothers) and offspring (chicks): we expect a positive relationship for mother-
156 female offspring telomere length at least in the control groups, where inheritance has not been
157 modulated by experimental treatments; c) effects of chick growth and age on telomere length in
158 chicks from eggs where we manipulated both yolk T or maternal antibodies to determine how these
159 two factors are interacting in the set-up of growth trajectories and immune response in offspring,
160 and how these in turn were related to telomere dynamics: based on the cost of growth hypothesis,
161 we expected telomeres of chicks at the end of growth to be shorter in males from the maternal LPS
162 treatment (MAb) group. Based on the trade-off between growth and immune system maturation,
163 chick and fledglings telomere lengths should be shorter in experimental groups, except for males of
164 the testosterone group that should have longer telomeres when reaching sexual maturity.

165

166 **MATERIAL AND METHODS**

167 Full details of our experimental protocol and sampling design are given in (Torres et al., 2019). Zebra
168 finches were housed under controlled environmental conditions (temperature 19–23 °C; humidity
169 35–55%; constant light/dark schedule, 14L:10D, lights on at 07.00 AM) and fed with a mixed seed
170 (millet) diet, water, grit, and cuttlefish bone (calcium) provided *ad libitum*, and a multivitamin
171 supplement in the drinking water once per week. Experienced adult male and female birds > 90 days
172 of age (*i.e.* birds that had been paired or laid eggs previously) were randomly paired and housed in
173 individual breeding cages (51 cm x 39 cm x 43 cm), each with an external nest box (14 cm x 14.5 cm x

174 20 cm). Breeding pairs had access to 6 g of egg food supplement (20.3% protein: 6.6% lipid) per day
175 during paring, laying and chick rearing. All breeding pairs were checked daily for egg-laying to record
176 laying date, egg mass, and clutch size. Freshly laid eggs were weighed (\pm 0.001 g), and individually
177 numbered to identify laying order. Experiments and animal husbandry were carried out under a
178 Simon Fraser University Animal Care Committee permit (no. 1074B-94), in accordance with
179 guidelines from the Canadian Committee on Animal Care (CCAC).

180

181 Experimental procedure

182 **a. LPS treatment.** To manipulate potential costs of reproduction, and the level of maternal
183 antibodies in eggs, adult females (experimental, n = 41; control group, n = 38) were first immune
184 challenged before egg laying. Females in the experimental group were injected intraperitoneally with
185 0.01 mg of a lipopolysaccharide (LPS; *Escheriachia coli*, serotype 055:B5; Sigma) diluted in 0.1 mL of
186 phosphate buffered saline solution (PBS, concentration = 0.1 mg/mL, females in control group
187 injected with only PBS) to initiate a primary immune response and again 10 days later to obtain a
188 stronger, secondary immune response. Three days after the second immune challenge, male-female
189 pairs were established and birds allowed to breed as described above (1st egg laid 10 days after the
190 second LPS injection). To evaluate the effect of cost of reproduction on telomere length we blood
191 sampled adult females at the very beginning of the experiment before the LPS challenge (T1, n=32),
192 when the first egg was laid (T2, n=25) and at the end of chick growth 21 days after hatching (T3,
193 n=31). Plasma (Torres et al., 2019) and yolk (Gasparini et al., 2002) antibody levels have been
194 previously found to be increased following such an immune challenge.

195 **b. T-treatment.** We used a split design manipulating yolk testosterone within clutches of LPS-treated
196 and control females (*i.e.* producing high- and low-MAb eggs) using *in ovo* egg injection of T on day 3
197 after the eggs were laid as described in Torres et al. (2019). Eggs from each clutch were randomly
198 assigned to either the testosterone or the control group (to control for variation among females and
199 genetic background). Eggs in the T-treated group were injected with 500 pg of testosterone (T, Fluka)

200 dissolved in 2 μ l of sesame oil (von Engelhardt et al., 2006). Before injection, the side of the egg was
201 cleaned with 100% ethanol, and the egg was held vertically with the apex at the top and the cap (air
202 cell) at the bottom, until the yolk floats to the top of the egg. The vehicle was injected into the yolk
203 using a 10- μ l removable needle Hamilton Syringe (gastight 1700 series) and 26½ G small hub
204 removable needle with a bevel tip. To reach the yolk, eggs were candled with a high-powered LED
205 flashlight (900 lumens) and the needle was pushed through the shell at an upward angle. The hole in
206 the shell was closed with a drop of cyanoacrylate glue (Loctite gel control) and eggs were placed back
207 in their nest once the glue was dry (ca. 10 minutes). Eggs in the control group were injected with 2 μ l
208 of sesame oil, but otherwise were treated in a similar way to eggs in the T-treatment.

209 At hatching nests were monitored daily to identify hatching order. Hatchlings were marked
210 by uniquely clipping down plumage for individual identification and at 8-12 days post-hatching all
211 birds were banded with a numbered aluminium ring. Body mass (\pm 0.01 g) and the length of tarsus
212 were recorded (\pm 0.01 mm) at hatching (day 0) and independence (30 days post-hatching). Juveniles
213 (30 days of age) were then removed from their natal cages and were housed in same-sex communal
214 cages with visual and acoustical contact with birds of the opposite sex, until sexual maturity at 90
215 days of age. Chicks were sexed based on their sexually dichromatic plumage. To compare telomere
216 length of offspring and mothers, and effects of LPS/T-treatments and growth on chick telomere
217 length, we used blood samples from chicks at the end of their growth period (day 26) and again close
218 to sexual maturity on day 82 (Torres et al., 2019).

219

220 **Telomere length assay using quantitative Polymerase Reaction Chain (qPCR)**

221 Telomere length was measured on DNA obtained from frozen red blood cells of adult females and
222 chicks of zebra finch. Red blood cells of birds are nucleated and DNA can be easily extracted
223 (Nucleospin Blood QUIckPure kit, Macherey-Nagel) in adequate quantity (assessed by
224 spectrophotometric absorbance, Nanodrop 1000 Thermo Scientific) and quality (ratios A260/280 and
225 A 260/230 and by checking for DNA degradation after gel-migration on a sub-sample, see Electronic

226 Supplementary Material 1) for qPCR amplification. DNA was then diluted using sterile distilled water
227 at 5 ng/ μ L for amplification using telomere and control gene (GAPDH) primers previously designed
228 and used in the same species (Reichert et al., 2015a), with a BRYT Green fluorescent probe (GoTaq
229 qPCR Master Mix, Promega). The samples were amplified on a 384 wells thermocycler (CFX-384,
230 Biorad Hercules), in a final volume of 10 μ L containing 200 nM of primers forward and reverse (0.4 μ L
231 of each diluted at 5 μ M), 5 μ L of SYBER Green PCR mix (Promega), 2 μ L of pre-prepared diluted DNA
232 (*i.e.* 2 ng) and 2.2 μ L of sterilised distilled water. The amplification conditions were set as 95°C for 2
233 min, followed by 40 cycles of data collection at 95°C for 15 s, 56°C for 30 s and 72°C for 30 s for
234 GAPDH. The conditions for the telomere sequences' amplification were set at 95°C for 2 min
235 followed by 30 cycles of data collection for 15 s at 95°C, 30 s at 56°C, and 1 min at 72°C. Each
236 amplification ended by a melting curve to check for non-specific signals. On each plate, a dilution
237 curve was run, using a randomly chosen sample serially diluted from 20 to 1.25 ng/ μ L, to establish
238 the efficiency of the qPCR amplification. Because amplification temperatures were different for the
239 telomere and the control gene (56°C and 60°C), the runs were conducted on different plates. Thus,
240 our samples were measured (in duplicates) using 2 runs of 2 plates (telomere and control gene).
241 Efficiencies of the qPCR were of 99.3 and 100.3% (telomere) and 100.6 and 99.1% (control gene).
242 Telomere length was finally calculated by (Pfaffl, 2001) and expressed as the T/S ratio (roughly the
243 ratio between telomere and control gene amplifications, corrected for amplification efficiencies,
244 thereafter z-transformed, see below). Intra-class coefficients for T/S ratio estimating intra-run
245 repeatability is of 0.989, and of 0.901 for inter-run repeatability (based on 10 repeated samples over
246 the 2 runs).

247

248 **Statistical analysis**

249 We used a mixed model approach, as describe in the following steps:

250 (i) for adult females, we used generalized linear mixed models (using females ID as random
251 factor) to test how telomere length was influenced over 1) the egg production period (T1 as initial

252 length and T2 as final length), 2) the chick rearing period (T2 to T3) and then we verify how this is
253 corroborated by statistical analysis over 3) the entire reproduction period (T1 to T3). Repeated
254 female telomere length values (at T1, T2 and T3) were used as response variable. As fixed factors,
255 time (T1, T2, T3), clutch size or brood size at hatching and fledgling, and female (maternal) treatment
256 (LPS injection) were used, as well as the interaction term Time x LPS-treatment which was kept in the
257 final models since we were interested in the timing of the putative effect of maternal treatment on
258 telomeres. Using the methods of (Simons et al., 2014), we distinguished telomere elongation from
259 measurement error within our longitudinal data.

260 (ii) resemblance of mother-offspring telomere lengths was tested using a generalized linear
261 mixed model with telomere length of chicks as the response variable and mother telomere length,
262 chick sex and the interaction as fixed factors, and nest ID as a random factor. Models were
263 successfully run to test the mother-offspring telomere length relationship at different ages (mother
264 telomere length at egg laying, end of chick rearing, chick telomere length at day 26, day 82) and
265 within experimental groups (control, LPS, all groups together).

266 (ii) for chicks, we used a generalized linear mixed model to test for the influence of maternal
267 LPS treatment (MAb) and T-treatment (testosterone) on telomere length at fledging (day 26, Nest
268 identity was included as a random factor to control for chicks raised in the same nest), or on
269 telomere lengths measured at fledging and at sexual maturity (day 26 – day 82). We kept these two
270 separated analyses in the manuscript since we were interested in the effect of our experimental
271 treatments on telomere length during growth and during sexual maturity. In that case, Nest identity
272 was included as a random factor as well as chick Age (day 26 – day 82), but none of the Age
273 interactions were kept in the final model since any were showing strong effects and also to reduce
274 the number of fixed factors included, due to sample size constrains. The following fixed factors were
275 included: chick sex, brood size at fledging, growth rate in body mass (g/day), maternal treatment
276 (LPS) and T treatment (testosterone injection). Growth rate, brood size at fledging (model at fledging)
277 and the interaction terms concerning maternal x T treatments x chick sex (model until adulthood)

278 were conserved in the final models, since we were looking for synergistic effects between treatments
279 and we knew that males from LPS mothers were bigger at 30 days (Torres et al., 2019).

280 All variables were z-transformed beforehand (Verhulst, 2019), analyses were performed on R
281 v.3.5.1., and standardized marginal effects plot with 95% confidence intervals were obtained using
282 the 'sjPlot' package in R (Lüdecke, 2017). Maternal investment was evaluated by computing a
283 principal component analysis (PCA) using clutch size, brood size at hatching and brood size at fledging
284 (see ESM) to get independent variables on maternal investment in reproduction that can be used in
285 the mixed model testing variation in female telomere lengths over the entire reproduction period
286 (i.e. that encompasses egg production and chick rearing). We checked for variance inflation factor
287 (VIFs, (Zuur et al., 2010)) when using in the same model maternal investment in egg production
288 (PCA2) and in chick rearing (PCA1). Results were interpreted based on significance (P value < 0.05)
289 and on effect sizes and their 95% intervals using benchmarks proposed by (Nakagawa and Cuthill,
290 2007) ($r=0.1, 0.3, 0.5$ correspond to small, medium and large effects). We present models containing
291 more explanatory variables with the lower AICc values.

292

293

294

295 RESULTS

296 Telomere lengths of adult females as a cost of reproduction

297 Telomere length of adult females at egg laying (T1) was found to be negatively affected by clutch size
298 and LPS treatment, the interaction Clutch size*LPS-treatment having an effect size of 0.66 (Table
299 2A). Regression analysis by treatments suggested that clutch size explained telomere length variation
300 in control females ($r^2 = 0.45$, estimates: -1.483 ± 0.492 , $t = -0.381$, $P = 0.012$), but not in LPS females
301 ($r^2 = 0.01$, estimates: -0.180 ± 0.474 , $t = -0.381$, $P = 0.711$). A similar trend was found for changes in
302 telomere length of females over the egg production period (measured at T1 and T2, Table 2B, Figure
303 1A). Again, the Clutch size x LPS interaction had an effect size = 0.45, underlying that clutch size was
304 negatively related to female telomere lengths in control ($r^2 = 0.18$, estimates: -1.048 ± 0.357 , $t = -$
305 2.937 , $P = 0.006$), but not in LPS females ($r^2 = 0.01$, estimates: -0.176 ± 0.317 , $t = -0.557$, $P = 0.582$).
306 Time had a small effect size, *i.e.* no significant changes in telomere lengths were observed over the
307 egg production period in adult females.

308 For female telomere dynamics during the chick rearing period (*i.e.* from the egg stage to day
309 25 post-hatching), females challenged with LPS before pairing lost significantly less telomere length
310 during the rearing period than control females (Table 2C, Time x LPS treatment, $r^2 = 0.05$, estimates: $-$
311 0.340 ± 0.276 , $t = -1.233$, $P = 0.228$; control females, $r^2 = 0.314$, estimates: -1.157 ± 0.343 , $t = -3.379$,
312 $P = 0.002$) and had on average shorter telomeres. Change in telomere length (T2 –T3) was even
313 found to be positive in LPS females (Figure 1B) though lengthening was not significant (comparison of
314 variance test, $F = 0.935$, $P = 0.795$). Results are consistent when controlling for clutch size instead of
315 brood size at fledging (LPS treatment, $P = 0.015$; Time x LPS treatment, $P = 0.010$). Again, Time had a
316 small impact *per se* on adult females' change in telomere length (Table 2C).

317 Considering telomere dynamics over the whole reproduction period (egg production plus
318 chick rearing periods, T1 – T3), laying and rearing investments had the largest effects on female

319 telomere length, both being negatively related to telomere length Table 2D). While effects of female
320 treatment were not significant, there was still a moderate effect size of the LPS treatment x Rearing
321 investment but regressions were non-significant in LPS ($r^2 = 0.02$, estimates: -0.139 ± 0.156 , $t = -$
322 0.886 , $P = 0.380$) or control females ($r^2 = 0.07$, estimates: -0.468 ± 0.279 , $t = -1.679$, $P = 0.101$, Figure
323 1C). Time and LPS treatment x Laying investment were not found to have important effects on
324 telomere lengths (Table 2C).

325

326 **Mother-chick telomere length resemblance**

327 Correlation was found significant only when both telomere lengths were compared at the
328 fledging stage and in the control group (13 females, 19 chicks; all other associations: $0.445 > P >$
329 0.846). There was a chick sex effect (males having shorter telomeres than females, estimates $-1.11 \pm$
330 0.46 , $t = -2.43$, $P = 0.028$; Effect sizes: -0.86 (CI₉₅ $[-1.12, -0.12]$) and the interaction term Chick sex x
331 Mother TL was significant (estimates -0.77 ± 0.36 , $t = -2.15$, $P = 0.048$; Effect sizes: -0.62 (CI₉₅ $[-1.65, -$
332 $0.08]$). This suggests that adult females with longer telomeres at fledging raised female offspring with
333 longer telomeres (Linear regression, estimates 0.55 ± 0.03 , $t = 16.04$, $P < 0.001$), while the
334 relationship was non-significant for males (estimates -0.21 ± 0.22 , $t = -0.93$, $P = 0.373$, Figure 2).
335 There was a marginally significant positive effect of mother telomere length: estimates 0.56 ± 0.30 , t
336 $= 1.86$, $P = 0.083$, random effect *Nest ID*, 0.010 ± 0.098 . This denotes a large effect of mother
337 telomere length on fledging telomere length (Effect sizes: 0.75 (CI₉₅ $[-0.04, 1.54]$). There was no
338 significant effect of brood size at fledging with a moderate positive effect size on chick telomere
339 length (estimates 0.38 ± 0.13 , $t = 1.86$, $P = 0.088$; Effect sizes: 0.38 (CI₉₅ $[-0.02, 0.50]$).

340

341 **Telomere dynamics during growth in chicks**

342 *Effects of T treatment (yolk testosterone) and LPS treatment (maternally derived antibodies (MAb))*
343 *on fledging TL*

344 At day 25, the largest effects on fledging telomere length were those of LPS treatment, the LPS-
345 treatment* offspring sex interaction and with T-treatment (Table 3). T-treatment had a small
346 negative effect on fledging telomere length but the interaction with sex was moderate and positive.
347 The three-way interaction LPS x T-treatment x sex was large and negative: testosterone egg injection
348 had a positive impact on telomere length of fledgling but in females in the LPS group and in males in
349 the control group (Figure 3A).

350 *Effects of egg and LPS treatments on telomere dynamics to sexual maturity*

351 There was a significant but small negative effect of age on offspring telomere length from hatching to
352 adulthood (Table 4). Additional factors had effects of similar small negative impact while being non-
353 significant: offspring sex (males having shorter telomeres, and T-treatment. LPS treatment had a
354 stronger, moderate negative effect, as did the interaction terms with sex and T-treatment (LPS
355 treatment x sex, LPS x T treatments). There was a moderate positive effect of the T-treatment x Sex .
356 The three-way interaction term LPS x T treatments x Sex had a medium negative effect on offspring
357 telomeres from fledging to adulthood: female offspring from testosterone-treated eggs produced by
358 LPS-treated mothers tended to have longer telomeres than those hatched from control-eggs
359 produced by LPS-treated mothers; in males, offspring from testosterone-treated eggs and control
360 mothers had longer telomeres than those from testosterone-treated eggs produced by LPS-treated
361 mothers (Figure 3B).

362

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364

365 **DISCUSSION**

366 Here we investigated effects of reproduction on adult telomere dynamics, the relationship between
367 mother-offspring telomere length, and effects of growth and maturation on chick telomere dynamics
368 using an experimental approach where we manipulated maternal immune function at laying, and
369 maternally-derived MAb and testosterone *in ovo* [31]. We found that producing large clutches and
370 rearing chicks were generally associated with a cost of telomere loss in adult females, but this
371 relationship was weaker in LPS-treated females. Adult females with longer telomeres raised female
372 offspring with longer telomeres at fledging, but telomere length of male offspring was independent
373 of maternal telomere length. In chicks, female offspring from testosterone-treated eggs produced by
374 LPS-treated mothers tended to have longer telomeres than those hatched from control-eggs and
375 produced by LPS-treated mothers whereas in males, offspring from testosterone-treated eggs and
376 control mothers had longer telomeres than those from testosterone-treated eggs produced by LPS-
377 treated mothers.

378

379

380 **Adult female telomere shortening as a cost of reproduction**

381 Telomeres are hypothesized to shorten when individuals undergo energy-demanding
382 activities (reviewed in (Angelier et al., 2019)) and in particular reproductive activities (Bichet et al.,
383 2020; Reichert et al., 2014; Sudyka et al., 2019). There is a global consensus that higher reproductive
384 success is achieved at the expense of an accelerated attrition of parental telomeres, both from
385 longitudinal (Bauch et al., 2013; Sudyka et al., 2019) and experimental (*e.g.* (Reichert et al., 2014))
386 studies. However, of seven studies that have experimentally tested the deleterious impact of
387 reproduction on telomeres in vertebrates and invertebrates, only four confirmed that reproduction
388 decreased telomere length (Sudyka, 2019). This suggests either that (i) parents may escape telomere
389 loss as a cost of reproduction in certain conditions or (ii) that only specific stages of the reproduction

390 are costly for the parents. For instance, in common terns, telomere erosion is observed in parents
391 only if their chicks do not die before the age of 10 days (Bauch et al., 2013; Bichet et al., 2020). The
392 negative relationship we report between clutch size and female telomere length at laying, between
393 telomere length of females over the reproduction period and laying/rearing investments confirm
394 that reproduction shorten adult telomeres in zebra finches. We have no direct evidence for the
395 mechanisms involved, and our observation might involve two non-exclusive explanations, *i.e.* energy
396 trade-offs and/or deleterious reproductive / stress hormones effects. Indeed, corticosterone levels
397 were observed to be higher in common terns with higher reproductive success but also shorter
398 telomeres (Bauch et al., 2016). Interestingly, our study suggests that females immune challenged
399 before laying (LPS-treated) did not shorten telomere length over reproduction as much as control
400 females did, while LPS-females did not produce lighter chicks (*i.e.* without apparently decreasing
401 their reproductive investment). There may be two nonexclusive explanations: (i) we cannot exclude
402 that LPS-treated females actually decreased their parental investment, protecting telomere length,
403 which could have been compensated by males. In fact, only LPS females showed negative values of
404 investment in chick rearing. A sex specific telomere cost of chick rearing for the parental sex most
405 concerned by chick provisioning was previously observed in males common terns (Bauch et al.,
406 2016). (ii) LPS females may have benefited from an energy trade-off biased towards body
407 maintenance because of the activation of the immune activity prior to egg production. Following the
408 concept of the hormetic response, a short term (*i.e.* weeks) adaptation to inflammation could be the
409 enhancement of antioxidant defences (Costantini, 2014). While the hypothesis that oxidative stress
410 shortens telomeres *in vivo* is still debated (Boonekamp et al., 2017; Reichert and Stier, 2017), a
411 recent experimental supplementation of tocopherol and selenium in white stork (*Ciconia ciconia*)
412 chicks suggested a telomere protective role of antioxidants (Bichet et al., 2020). Additional cell
413 mechanisms involved in the mitigation of deleterious effects of inflammation, like DNA repairing
414 (Calvo et al., 2012), may also have contributed to the reduced telomere loss in our LPS-females.

415

416 **Relationship between mother and offspring telomere lengths**

417 The mode of inheritance of telomere length and the relative importance of genetic versus
418 environmental determinants remains a hotly debated topic in telomere research (Dugdale and
419 Richardson, 2018). The genetic basis of offspring telomere length results from intertwined effects of
420 variation in telomere length of parental gametes, re-setting of the telomere length in the zygote at
421 fertilisation, effects of parental (most often paternal) age and epigenetic or epigenetic-like
422 inheritance (Bauch et al., 2019; Eisenberg, 2019; Entringer et al., 2018). In birds, telomere length
423 inheritance was first believed to involve stronger maternal than parental inheritance (Horn et al.,
424 2011; Reichert et al., 2015b), possibly related to a z-linked mechanism. However, accumulated data
425 so far has questioned this sex-specific inheritance in birds (Atema et al., 2015; Becker et al., 2015) or
426 in lizards (Olsson et al., 2011a). Additionally, environmental factors have additive effects on the
427 parental-offspring telomere length resemblance (*e.g.* (Becker et al., 2015; Voillemot et al., 2012) but
428 see (Belmaker et al., 2019). For instance in king penguins (*Aptenodytes patagonicus*), offspring
429 telomere length initially showed a large maternal influence (10 days after hatching) which eroded
430 over the growing period (Reichert et al., 2015b). We found a positive relationship between mother-
431 offspring telomere length but only at day 26 post-hatching. This is consistent with previous results in
432 birds (Belmaker et al., 2019; Horn et al., 2011; Reichert et al., 2015b), though we cannot definitively
433 test for genetic effects in the absence of paternal telomere length data, and the lack of relationship
434 at sexual maturation (day 82) also suggests additional environmental effects. However, our data
435 suggest a chick sex effect, with a positive relationship between maternal telomere length and that of
436 female, but not male, offspring. Whether there is sex-specific selection for longer telomeres in
437 female zebra finches as it has been observed in other vertebrates (Olsson et al., 2011b) is an open
438 question. It is important to note that a previous study of telomere heritability conducted in captive
439 zebra finches could not distinguish between maternal and paternal contributions to offspring
440 telomere length but suggested high additive genetic variance (Atema et al., 2015).

441

442 **Telomere length up to fledging in relation to growth, LPS and T-treatments**

443 Overall, our study showed that the experimental treatment of reproductive females (LPS challenge
444 prior to egg production) and of eggs (testosterone injection) modulated telomere length of chicks,
445 with a larger effect of LPS. More importantly, we found a large effect size for the interaction of both
446 treatments. We found that chick telomere length at the end of growth (day 26) was weakly affected
447 by T-treatment, but largely negatively affected by the LPS treatment of reproductive females, *i.e.*
448 chicks from eggs produced by LPS females had shorter telomeres. Since those chicks were not lighter
449 than control chicks at that stage, it is unlikely that a sub-optimal parental feeding and a stronger
450 growth-soma energy based trade-off could explain this. Indeed, a causal relationship between higher
451 growth rate and shorter telomeres in LPS chicks would be supported if LPS chicks were also
452 characterized by a higher growth rate over the rearing period. Such an increased growth rate was
453 found but only in male chicks (Torres et al., 2019) and a negative link between growth rate and
454 telomere length was observed in female chicks in our current study. Therefore, a non-energy related
455 explanation may be more suitable to explain our sex-specific pattern of telomere length variation in
456 chicks in relation to maternal LPS treatment. Since we are measuring chick telomere length in blood
457 cells (including a small proportion of white blood cells), MAb-derived from LPS treatment may have
458 prematurely triggered the activation of the chicks' immune system accelerating telomere erosion of
459 white blood cells (*i.e.* increased cell division rate). This may have driven to variation in the ratio
460 white/red blood cells which may account for LPS-induced changes in mean relative telomere length,
461 either due to large differences in telomere lengths among white vs. red blood cell populations
462 (Olsson et al., 2020) or to a higher division rate of white blood cells leading to faster telomere
463 shortening. Such a pre-activation of the chick immune system (*i.e.* in anticipation of infection) primed
464 by LPS-antibodies from mothers and involving macrophages may also be associated with increased
465 immune-derived oxidative stress (Emre et al., 2007), putatively also deleterious for telomeres. An
466 alternative explanation is that the observed pattern is due to the effect of social stress on chick
467 telomeres, following a change in maternal behaviour as LPS immunisation may decrease nestling

468 feeding activity by the mother (Bonneaud et al., 2003). Such a stressful environment due to
469 increased nestling competition is known to accentuate telomere shortening in starlings (Nettle et al.,
470 2015). Both MAb and nestling stress explanations match with short-term effects of LPS on fledging
471 telomere length.

472

473 **Telomere length to sexual maturity and antagonistic effects of LPS and T-treatments**

474 We found some evidence for antagonistic effects of LPS-induced MAb and T-treatment on chick
475 telomeres at both stages of development either when considering telomere length at day 26 or as a
476 repeated variable (day 25 – day 82). This suggests that cross talk may exist between these two
477 components in birds (Tobler et al., 2010). Since those effects were similar at fledging (at day 26) and
478 at adulthood (day 82), it suggests that the impact of our experimental treatments during the main
479 growth period had persistent impacts on chick telomeres, *i.e.* they were not subsequently modulated
480 during sexual maturation. Interestingly, we observed a sex-specific impact of T-treatment on fledging
481 telomeres in relation to the mother LPS exposure before egg laying. Testosterone mitigated the
482 negative effect of LPS-treatment observed in female fledglings but also negatively affected telomere
483 length of female offspring hatched from control eggs, while it had a positive effect on telomere
484 length in male offspring hatched from control eggs and no effects on LPS-hatched males. We
485 currently have no explanation for these results but they suggest potentially very complex
486 interactions between maternal effects, sex and telomere dynamics.

487 Evidence for synergistic/antagonistic effects of MAb and yolk T treatments on telomere
488 dynamics contrast with the general lack of interaction effects on chick growth and other phenotypic
489 traits reported previously (Torres et al., 2019). The only correspondence between our two sister
490 studies concerns the phenotype and telomere length of male offspring raised by control-mothers and
491 hatched from testosterone-injected eggs: they presented both longer telomeres and a higher
492 breeding quality (Ardia et al., 2010), which matches well with the telomere quality hypothesis
493 (Angelier et al., 2019): individuals with longer telomeres are those that may insure a more efficient

494 body maintenance but also have the highest breeding success. Whether testosterone allows chicks to
495 divert energy from costly physiological traits (immunity) towards higher investment in reproduction
496 or simply improves the resilience of individuals to stress (social stress) need to be tested. Because
497 pre-natal exposure to testosterone has been shown to induce an oxidative cost in male nestling of
498 zebra finch (Tobler and Sandell, 2008), the mitigating effect of testosterone on telomeres may not be
499 mediated by an improved oxidative balance in our birds, but rather from better access to food
500 resources (e.g. due to higher begging activity in testosterone male chicks (von Engelhardt et al.,
501 2006)) and/or an indirect social effect like dominance. However, the fact that telomere dynamics are
502 maintained from fledging to adult sexual maturity also suggests that individual differences in
503 telomere length are established early in life. It would be interesting to test how our treatments are
504 modulating the telomere length response of our individuals during their future reproduction or when
505 they will face stressful events.

506

507

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510 **COMPETING INTERESTS**

511 The authors declare no competing of financial interests

512

513 **AUTHORS' CONTRIBUTIONS**

514 T.D.W. and R.T. collected the data and R.T. run all the physiological analyses, F.C. extracted the DNA
515 and S.Z. ran the qPCR measurements of telomere length, S.Z. and F.C. analysed the qPCR data, F.C.
516 and T.D.W. did the statistical analyses, and drafted the final manuscript on which R.T. made
517 comments.

518

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525

526 **DATA ACCESSIBILITY**

527 Data will be deposited on Figshare once the paper is accepted.

528

529

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771 **FIGURE LEGENDS**

772 Figure 1: Change in telomere length of adult female zebra finches from the control or the
773 experimental treatment (LPS challenge before pairing), during the **(A)** egg production period in
774 relation to clutch size; **(B)** over the chick rearing period (T2 and T3 refer to times of sampling of adult
775 females, see Experimental procedure); **(C)** the whole reproduction period in relation to investment in
776 chick rearing (PCA1 axis including brood sizes at hatching and at fledging, see statistics). Positive
777 changes were not found to reflect true telomere lengthening and regressions were not significant in
778 1C (see Results). Telomere length is expressed as z-transformed TS ratio and 0 is then an individual
779 value equal to the mean population value.

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781 Figure 2: Mother-offspring linear relationships of z-transformed telomere lengths (T/S ratio)
782 measured both at the fledging stage. Only adult females from the control were taken into account,
783 which encompassed 13 females and 19 chicks (6 females and 13 males).

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785 Figure 3: Box plot of mixed model predicted values of **(A)** fledging telomere length (day 25) and **(B)**
786 fledging telomere lengths (repeated values at day 25 and 82, sexually mature adults) in relation to
787 egg (testosterone injection) and mother (LPS challenge) experimental treatments. C indicates control
788 groups for both treatments. Results are separated by chick sex. Means value \pm SE are represented
789 using open circles. Telomere lengths are expressed as z-transformed TS ratio and 0 is then an
790 individual value equal to the mean population value.

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800 TABLES

801 Table 1. Predictions for effects of experimentally induced maternal effects (LPS) and egg-yolk content
802 (testosterone) on chick telomere length after embryonic development and post-hatching growth
803 (day 26) and young adult final development (day 82) in zebra finch. Those predictions are based on
804 our previous sister paper (Torres et al., 2019) main findings (indicated in grey) that LPS-induced
805 maternal effect accelerated growth rate in male chicks at the end of post-hatching growth, and that
806 immune response at the end of post-hatching growth (egg-yolk treated chicks) and at the end of
807 young adult maturation period (chicks produced by LPS treated mothers) was enhanced. Males of
808 the testosterone group that were found of better pairing quality should have longer telomeres when
809 reaching sexual maturity. Predictions either followed the trade-off / telomere hypothesis. Table
810 modified from Torres *et al.*, (Torres et al., 2019).

811

Treatment	Sex	Immature phenotype (day 26)			Young adult phenotype (day 82)		
		Growth	Immune function	Telomere length	Immune function	Adult quality	Telomere length (days 26-82)
Yolk testosterone (T)	M	0	+	-	0	+	+
	F	0	+	-	0	0	0
LPS-maternally derived antibodies (LPS)	M	+	0	-	+	0	-
	F	0	0	0	+	0	-
Interaction T x LPS	M	0	0	0	0	0	0
	F	0	0	0	0	0	0

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815 Table 2. Output of the linear model (2A) and generalized linear mixed models (2B, C, D) testing the
 816 effects at egg laying (A) and of egg production (B, T1 and T2), chick rearing (C, T2 and T3) and
 817 reproduction investment in both periods (D, T1 and T3) on adult female telomere lengths. Females
 818 belong either to the control (sham injected, n = 12) or LPS-challenged (n = 13) group which was
 819 conducted before the formation of the breeding pairs. Telomere lengths were measured before the
 820 LPS challenge (T1), when the first egg was laid (T2) and at the end of chick growth 21 days after
 821 hatching, and are expressed as z-transformed TS ratio and has been corrected for the regression to
 822 the mean effect. Laying and rearing investments are independent variables obtained from a principal
 823 component analysis using clutch size at laying (PC2, Laying investment), and brood sizes at hatching
 824 and at fledging (PC1, Rearing investment). Random effects are described using σ^2 and T_{00} statistics
 825 allowing to evaluate the intraclass correlation coefficient of the random factor (ICC). N_{id} indicate the
 826 number of levels for the random factor and the number of observations are also indicated. Marginal
 827 R^2 only includes the fixed factors variance while the Conditional R^2 takes fixed and random factors
 828 into account. While some ICC were close to 0 (similar within and between random factor variance),
 829 mixed modelling was always presented as Conditional R^2 was always higher than Marginal R^2 .

TABLE 2A. MOTHER TELOMERE LENGTH AT EGG LAYING			
Predictors	Effect size	CI 95%	P value
Intercept	0.22	-0.30 – 0.75	0.020
Clutch size	-0.80	-1.39 – -0.21	0.015
LPS treatment	-0.58	-1.13 – -0.03	0.050
Clutch size x LPS treatment	0.66	-0.04 – 1.36	0.078
<i>Observations</i>	28		
<i>Marginal / Conditional R²</i>	0.265 / -		
TABLE 2B. MOTHER TELOMERE CHANGES OVER EGG PRODUCTION			
Predictors	Effect size	CI 95%	P value
Intercept	0.12	-0.26 – 0.49	0.052
Clutch size	-0.57	-1.00 – -0.13	0.017
LPS treatment	-0.35	-0.75 – -0.05	0.099
Time	-0.09	-0.34 – 0.15	0.459
Clutch size x LPS treatment	0.45	-0.05 – 0.96	0.092
Random effects σ^2	1.09		
T_{00}	0.09		
ICC	0.08		

N_{id}	32		
<i>Observations</i>	58		
<i>Marginal / Conditional R^2</i>	0.121 / 0.188		
TABLE 2C. MOTHER TELOMERE CHANGES OVER CHICK REARING			
Predictors	Effect size	CI 95%	P value
Intercept	-0.07	-0.52 – 0.38	0.230
Brood size	0.01	-0.30 – 0.32	0.957
LPS treatment	-1.30	-2.29 – -0.30	0.016
Time	-0.20	-0.47 – 0.07	0.153
Time x LPS treatment	0.66	0.44 – 2.41	0.009
Random effects σ^2	0.38		
T_{00}	0.43		
ICC	0.53		
N_{id}	32		
<i>Observations</i>	57		
<i>Marginal / Conditional R^2</i>	0.077 / 0.566		
TABLE 2D. MOTHER TELOMERE CHANGES OVER REPRODUCTION			
Predictors	Effect size	CI 95%	P value
Intercept	0.00	-0.32 – 0.32	0.057
LPS treatment	-0.30	-0.71 – -0.08	0.170
Time	-0.01	-0.20 – 0.18	0.915
Laying investment	-0.39	-0.70 – -0.08	0.020
Rearing investment	-0.52	-0.92 – -0.12	0.018
LPS treatment x Laying investment	0.17	-0.13 – 0.46	0.281
LPS treatment x Rearing investment	0.46	-0.06 – 0.47	0.094
Random effects σ^2	0.86		
T_{00}	0.08		
ICC	0.08		
N_{id}	32		
<i>Observations</i>	89		
<i>Marginal / Conditional R^2</i>	0.125 / 0.197		

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833 Table 3. Output of the generalized linear mixed model testing the effects of female immune
 834 challenge before pairing (LPS treatment) and egg testosterone injection (T treatment) on chick
 835 telomere length at fledging (day 25). The statistical model account for growth rate of chicks,
 836 calculated as body mass gain over the 25 days post-hatching (g/day), and brood size at fledging. Nest
 837 ID was used as random effect. Telomere length is expressed as z-transformed TS ratio. See Table 2
 838 for Random effects description.

839

TABLE 3. FLEDGING TELOMERE LENGTH			
Predictors	Effect size	CI 95%	P value
Intercept	0.65	-0.18 – 1.47	0.125
LPS treatment	-0.87	-1.59 – -0.15	0.021
T treatment	-0.26	-0.78 – 0.27	0.343
Sex	-0.73	-1.68 – 0.22	0.136
Growth rate	0.22	-0.04 – 0.48	0.292
Brood size at fledging	-0.14	-0.40 – 0.12	0.114
LPS treatment x T treatments	0.65	-0.14 – 1.45	0.112
LPS treatment x Sex	0.79	0.06 – 1.52	0.038
T treatment x Sex	0.47	-0.15 – 1.08	0.141
LPS treatment x T treatment x Sex	-0.68	-1.46 – 0.10	0.095
Random effects σ^2	0.92		
T_{00}	0.08		
ICC	0.08		
N_{id}	31		
Observations	68		
Marginal / Conditional R^2	0.139 / 0.208		

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843 Table 4: Output of the generalized linear mixed model testing the effects of female immune
844 challenge before pairing (LPS treatment) and egg testosterone injection (T treatment) on chick
845 telomere lengths measured at fledging (day 25) and adult maturity (day 82). The statistical model
846 accounts for chick sex. LPS and T treatment refer to former mother challenge with LPS and
847 testosterone or sham injections in the egg. Chick ID was used as random effect. Telomere change and
848 length are expressed as z-transformed TS ratio. See Table 2 for Random effects description.

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TABLE 4. CHICK TELOMERE LENGTH OVER EARLY LIFE			
Predictors	Effect size	CI 95%	P value
Intercept	0.42	-0.19 – -1.04	0.001
Time	-0.29	-0.43 – -0.15	<0.001
Sex	-0.26	-0.63 – 0.11	0.180
LPS treatment	-0.47	-0.95 – 0.01	0.058
T treatment	-0.20	-0.61 – 0.20	0.331
LPS treatment x Sex	0.39	-0.12 – 0.91	0.137
T treatment x Sex	0.43	-0.04 – 0.90	0.080
LPS treatment x T treatments	0.40	-0.15 – 0.95	0.154
LPS treatment x T treatment x Sex	-0.46	-1.02 – 0.09	0.107
Random effects σ^2	0.64		
T_{00}	0.26		
ICC	0.29		
N_{id}	75		
Observations	137		
Marginal / Conditional R^2	0.131 / 0.385		

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