

## Supplementary Material

### **Intergenerational effects on offspring telomere length; interactions among maternal age, stress exposure and offspring sex**

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**Table S1.** GLMM modelling with Gaussian distribution to test whether prior maternal reproductive effort ((a) total number of eggs laid, or (b) total number of chicks reared up to the first three breeding events prior the old-mother breeding event at 3.5 years of age), maternal treatment, fathers' age at reproduction, and other selected fixed parameters (see "Data Analysis", Material and Methods) influenced early life telomere length (measured at ~ 30 days of age, values ln-transformed) in the offspring produced during the old-mother breeding event; female identity was added as random factor; \*indicates non-significant factor removed from the model ( $p > 0.05$ ). Number of offspring: 126; number of mothers: 52.

(a)

Parameter	Estimate	SE	df	t	p
Female ring identity (r)	0.021				
Residual	0.153				
Intercept	-0.491	0.305	48.240	-1.611	0.114
Maternal treatment (Challenging environment)	0.041	0.096	39.580	0.428	0.671
Father age at reproduction	<0.0001	<0.0001	41.330	0.196	0.846
Replicate (2)	-0.046	0.100	43.060	-0.457	0.650
Hatching order	<0.0001	0.031	115.200	0.003	0.998
Sex (Male)	0.061	0.075	118.700	0.810	0.420
Prior maternal egg laying effort	0.003	0.019	41.750	0.154	0.878
Offspring Body mass*					0.9

(b)

Parameter	Estimate	SE	df	t	p
Female ring identity (r)	0.021				
Residual	0.153				
Intercept	-0.396	0.207	58.690	-1.918	0.060
Maternal treatment (Challenging environment)	0.024	0.095	39.480	0.257	0.798
Father age at reproduction	<0.0001	<0.0001	40.330	0.259	0.797
Replicate (2)	-0.049	0.098	42.830	-0.497	0.622
Hatching order	0.003	0.031	116.100	0.083	0.934
Offspring sex (Male)	0.056	0.076	118.600	0.747	0.457
Prior maternal chick rearing effort	-0.011	0.021	44.800	-0.527	0.601
Offspring Body mass*					0.9

**Table S2.** (a) GLMM modelling with a Gaussian distribution to test the effects of maternal age (young or old, 6 month or 3.5 years of age respectively), maternal treatment (control environment or challenging environment), offspring sex, and selected fixed parameters (see “Data Analysis”, Material and Methods) on offspring body mass at nutritional independence (~ 30 days of age); female identity was added as random factor. Fixed factor estimates are indicated in parenthesis; r indicates random factor and its associated variance. The non-significant interactions ( $*p > 0.05$ ) were removed from the final model, significant factors are in bold. In (b) the same model as in (a) was performed using only the data from offspring produced by experimental females that bred at both breeding events.

(a)

Parameter	Estimate	SE	df	t	p
Female ring identity (r)	0.631				
Residual	0.942				
<b>Intercept</b>	<b>15.232</b>	<b>0.234</b>	<b>337.464</b>	<b>65.072</b>	<b>&lt;0.0001</b>
<b>Maternal age (old)</b>	<b>-0.420</b>	<b>0.131</b>	<b>415.380</b>	<b>-3.199</b>	<b>0.001</b>
Maternal treatment (Challenging environment)	-0.176	0.155	173.151	-1.130	0.260
Replicate (2)	-0.156	0.158	174.240	-0.987	0.325
Hatching order	-0.023	0.050	324.012	-0.459	0.647
Offspring sex (Male)	-0.148	0.104	345.678	-1.422	0.156
<b>Brood size</b>	<b>-0.204</b>	<b>0.060</b>	<b>420.947</b>	<b>-3.399</b>	<b>0.001</b>
Maternal treatment x Offspring sex*					0.8
Maternal treatment x Maternal age*					0.1
Maternal age x Offspring sex*					1.0
Maternal treatment x Maternal age x Offspring sex*					0.5

(b)

Parameter	Estimate	SE	df	t	p
Female ring identity (r)	0.528				
Residual	1.052				
<b>Intercept</b>	<b>14.475</b>	<b>0.449</b>	<b>95.708</b>	<b>32.206</b>	<b>&lt;0.0001</b>
Maternal age (old)	-0.120	0.161	147.352	-0.750	0.455
Maternal treatment (Challenging environment)	-0.174	0.288	40.693	-0.602	0.551
Replicate (2)	0.098	0.304	40.796	0.320	0.750
Hatching order	-0.017	0.078	146.588	-0.225	0.822
Offspring sex (Male)	-0.072	0.162	150.828	-0.443	0.659
Brood size	-0.137	0.085	176.518	-1.598	0.112
Maternal treatment x Offspring sex*					0.3
Maternal treatment x Maternal age*					0.1
Maternal age x Offspring sex*					0.8
Maternal treatment x Maternal age x Offspring sex*					0.5

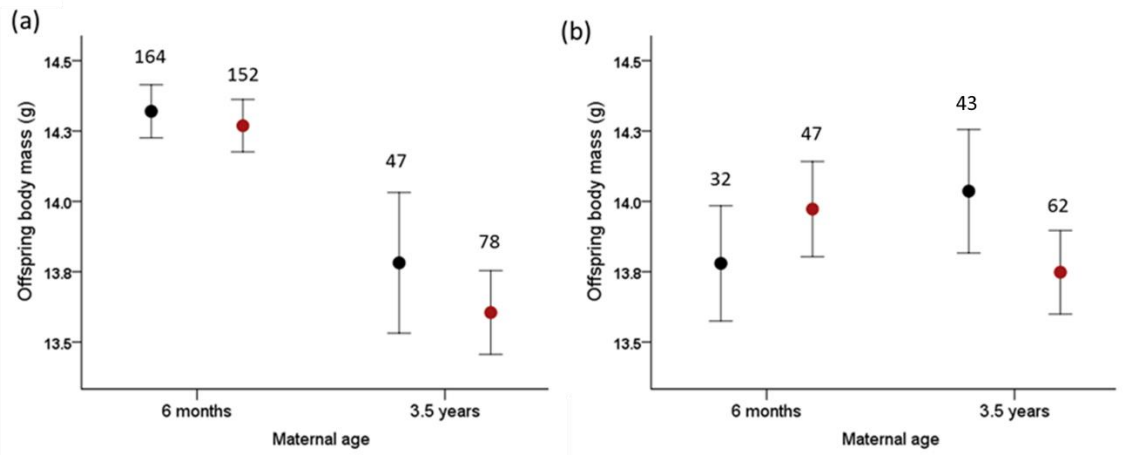
**Table S3.** (a) GLMM modelling with a Gaussian distribution to test the effects of maternal age (young or old, 6 month or 3.5 years of age respectively), maternal treatment (control environment or challenging environment), offspring sex, and selected fixed parameters (see “Data Analysis”, Material and Methods) on offspring telomere length measured at ~ 30 days of age (ln-transformed values); female identity was added as random factor. Fixed factor estimates are indicated in parenthesis; r indicates random factor and its associated variance. The non-significant factors ( $*p > 0.05$ ) were removed from the final model; significant factors are in bold. In (b) the same model as in (a) was performed using only the data from offspring produced by experimental females that bred at both breeding events.

(a)

Parameter	Estimate	SE	df	t	p
Female ring identity (r)	0.006				
Residual	0.202				
<b>Intercept</b>	<b>0.203</b>	<b>0.085</b>	<b>358.831</b>	<b>2.401</b>	<b>0.017</b>
<b>Maternal age (old)</b>	<b>-0.481</b>	<b>0.116</b>	<b>392.819</b>	<b>-4.145</b>	<b>&lt;0.0001</b>
Maternal treatment (Challenging environment)	-0.063	0.073	407.101	-0.872	0.384
Replicate (2)	-0.0814	0.0468	179.522	-1.740	0.084
Hatching order	-0.013	0.022	402.148	-0.613	0.540
Offspring sex (Male)	0.068	0.071	415.943	0.952	0.342
Brood size	-0.010	0.022	361.482	-0.444	0.657
<b>Maternal treatment x Offspring sex</b>	<b>-0.262</b>	<b>0.103</b>	<b>418.401</b>	<b>-2.545</b>	<b>0.011</b>
Maternal treatment x Maternal age	-0.026	0.150	402.226	-0.175	0.861
Maternal age x Offspring sex	-0.130	0.152	424.265	-0.857	0.392
<b>Maternal treatment x Maternal age x Offspring sex</b>	<b>0.461</b>	<b>0.199</b>	<b>430.575</b>	<b>2.311</b>	<b>0.021</b>

(b)

Parameter	Estimate	SE	df	t	p
Female ring identity (r)	0.012				
Residual	0.153				
Intercept	0.223	0.157	151.372	1.421	0.157
<b>Maternal age (old)</b>	<b>-0.358</b>	<b>0.142</b>	<b>164.605</b>	<b>-2.515</b>	<b>0.013</b>
Maternal treatment (Challenging environment)	0.038	0.145	169.338	0.265	0.791
Replicate (2)	-0.1547	0.0774	46.969	-1.998	0.051
Hatching order	-0.016	0.029	161.353	-0.563	0.574
Offspring sex (Male)	0.246	0.144	166.811	1.700	0.091
Brood size	-0.028	0.029	163.263	-0.960	0.339
<b>Maternal treatment x Offspring sex</b>	<b>-0.473</b>	<b>0.186</b>	<b>167.086</b>	<b>-2.541</b>	<b>0.012</b>
Maternal treatment x Maternal age	-0.153	0.183	166.252	-0.837	0.404
Maternal age x Offspring sex	-0.348	0.191	173.492	-1.816	0.071
<b>Maternal treatment x Maternal age x Offspring sex</b>	<b>0.648</b>	<b>0.249</b>	<b>173.256</b>	<b>2.606</b>	<b>0.010</b>



**Figure S1.** (a) Body mass at nutritional independence (~30 days old) of zebra finch offspring produced by mothers that bred during the young-mother breeding event at 6 months of age (89 control and 83 challenged mothers) and/or during the old-mother breeding event at 3.5 years of age (20 control and 32 challenged mothers). In (b) data are shown only from the subset of females that produced offspring during both the young- and old-mother breeding event (18 control and 26 challenged mothers). Data are shown as means  $\pm$  SE; black circles indicates offspring produced by control mothers and red circles indicates offspring produced by challenged mothers, numbers indicates offspring sample sizes separately by maternal treatment and maternal age.