No evidence for sex-specific effects of the maternal social environment on offspring development in Japanese quail (*Coturnix japonica*)

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Abstract

The social environment of reproducing females can cause physiological changes, with consequences for reproductive investment and offspring development. These prenatal maternal effects are often found to be sex-specific and may have evolved as adaptations, maximizing fitness of male and female offspring for their future environment. Female hormone levels during reproduction are considered a potential mechanism regulating sex allocation in vertebrates: high maternal androgens have repeatedly been linked to increased investment in sons, whereas high glucocorticoid levels are usually related to increased investment in daughters. However, results are not consistent across studies and therefore still inconclusive. In Japanese quail (*Coturnix japonica*), we previously found

25   that pair-housed females had higher plasma androgen levels and tended to have higher plasma

26   corticosterone levels than group-housed females. In the current study we investigate whether these

27   differences in maternal social environment and physiology affect offspring sex allocation and

28   physiology. Counter to our expectations, we find no effects of the maternal social environment on

29   offspring sex ratio, sex-specific mortality, growth, circulating androgen or corticosterone levels. Also,

30   maternal corticosterone or androgen levels do not correlate with offspring sex ratio or mortality. The

31   social environment during reproduction therefore does not necessarily modify sex allocation and

32   offspring physiology, even if it causes differences in maternal physiology. We propose that maternal

33   effects of the social environment strongly depend upon the type of social stimuli and the timing of

34   changes in the social environment and hormones with respect to the reproductive cycle and meiosis.

35

Keywords

37   fetal programming, steroids, development, mortality, stress response, sex allocation

38

1. Introduction

40   Variation in the social environment affects female behaviour and physiology with potential

41   consequences for reproductive investment. Changes in reproductive investment, in turn, modify the

42   prenatal environment of the developing offspring and can thereby profoundly shape offspring's

43   future phenotype (Groothuis et al., 2005; Guibert et al., 2010; Kaiser and Sachser, 2009, 2005). Pre-

44   and postnatal maternal effects of the social environment can bias offspring sex ratios (Clutton-Brock

45   and Iason, 1986; Michler et al., 2013; Minias et al., 2014) and affect offspring development and

46   behaviour, often in a sex-specific way (Kaiser and Sachser, 2009, 2005). Sex-specific maternal effects

47   are thought to have evolved as adaptations, maximizing fitness of male and female offspring for their

48   anticipated environment. Maternal steroid hormones provide important candidate signals,

49   transmitting effects of the social environment across generations. Hormones, and other compounds,

are transferred to the ovum and embryo and can profoundly affect offspring behavioural and physiological development (Groothuis et al., 2005; Groothuis and Schwabl, 2008; Kaiser and Sachser, 2005; Meylan et al., 2012; Radder, 2007; von Engelhardt and Groothuis, 2011).

Oviparous species, such as reptiles and birds, are especially suitable to explore prenatal effects because the maternal and offspring environment can be independently manipulated. Previous studies on avian species have shown effects of the social environment on sex allocation (Michler et al., 2013; Minias et al., 2014). In the great cormorant (*Phalacorcorax carbo sinensis*), social density positively correlates with the proportion of male offspring within broods (Minias et al., 2014). In contrast, female great tits (*Parus major*) bred in areas with experimentally increased nesting densities or who experienced areas with high nesting density as juveniles produce female-biased broods in the following year, whereas females breeding in areas with decreased nesting densities or reared in areas of naturally lower nesting density produce male-biased broods (Michler et al., 2013). In many vertebrate species, changes in both primary and secondary offspring sex ratio have been linked to variation in maternal plasma steroids around conception (reviewed by Alonso-Alvarez, 2006; James, 2008; Krackow, 1995; Navara, 2013a; Pike and Petrie, 2003). In avian species, increased levels of maternal androgens usually lead to male-biased offspring sex ratios (Goerlich-Jansson et al., 2013; Goerlich et al., 2009; Pike and Petrie, 2005; Rutkowska and Cichoń, 2006; Veiga et al., 2004, but see Correa et al., 2011), whereas increased levels of maternal glucocorticoids often result in female-biased offspring sex ratios (Bonier et al., 2007; Goerlich-Jansson et al., 2013; Love et al., 2005; Pike and Petrie, 2006, 2005, but see Gam et al., 2011; Henriksen et al., 2013). In Japanese quail (*Coturnix japonica*), naturally increased maternal faecal corticosterone (CORT) metabolite concentrations and experimentally elevated maternal plasma CORT concentrations are associated with a female-biased primary sex ratio (Pike and Petrie, 2006). In contrast to the findings in other species, maternal plasma testosterone levels of Japanese quail have been related to both an unbiased (Pike and Petrie, 2006) as well as a female-biased offspring sex ratio (Correa et al., 2011). This indicates that results from experimental and correlational studies are still inconclusive.

In addition to affecting offspring sex ratio, the maternal social environment can affect offspring growth and survival, which may be mediated by changes in maternal circulating levels of androgens and CORT. For example, in American red squirrels (*Tamiasciurus hudsonicus*), increased offspring growth rates at higher social densities have been attributed to the effects of increased maternal CORT (Dantzer et al., 2013). In Japanese quail, social instability resulted in an increase in agonistic interactions and reduced offspring body mass at the age of 1-3 weeks, compared to stable social groups (Guibert et al., 2010). Such effects on growth may be due to increased maternal CORT because artificially increasing maternal circulating CORT reduced offspring growth in Japanese quail (Hayward and Wingfield, 2004). The maternal social environment and maternal hormones can also have sex-specific effects on offspring growth and survival. In guinea pigs (*Cavia aperea*), housing females individually during pregnancy decreased growth of daughters compared to daughters of group-housed females, whereas growth of sons was non-significantly increased (von Engelhardt et al., 2015). Artificially increasing maternal circulating testosterone in zebra finches (*Taeniopygia guttata*) reduced the hatching success of sons and increased the post-hatching survival of daughters (Rutkowska and Cichoń, 2006). Experimental elevation of maternal CORT in European starlings (*Sturnus vulgaris*) increased the mortality of male embryos, led to a female-biased sex ratio at hatching, and reduced early growth in males (Love et al., 2005).

Maternal effects on offspring growth and survival may be attributed to (sex-specific) modulation of offspring endocrine physiology (Groothuis et al., 2005; Groothuis and Schwabl, 2008; Kaiser and Sachser, 2005) since both growth and survival can relate to circulating hormone levels (e.g. Braasch et al., 2011; Brown et al., 2005; Goodship and Buchanan, 2006; Goutte et al., 2010; Groothuis and Ros, 2005; Hull et al., 2007; Müller et al., 2009; Ros, 1999; Wada and Breuner, 2008). Studies on transgenerational effects of the maternal social environment on offspring physiology are scarce, especially in birds. However, in Japanese quail, maternal social instability increases the offspring's emotional reactivity scored in different behavioural tests, suggesting possible effects on the hypothalamic–pituitary–adrenal axis (HPA-axis) regulating the release of CORT (Guibert et al.,

102   2010). This assumption is corroborated by studies on guinea pigs, which even find sex-specific effects

103   of the maternal social environment on the HPA-axis in the offspring (Kaiser and Sachser, 2001; von

104   Engelhardt et al., 2015).

105   In our previous study on Japanese quail, we have shown that the social environment during

106   breeding affects female physiology (Langen et al., 2017). Females housed in pairs (one male, one

107   female) had higher plasma androgen concentrations and tended to have higher plasma CORT

108   concentrations than females housed in groups (one male, three females; see Langen et al. 2017 for

109   more details). Here, we examined the offspring of those females to investigate whether the maternal

110   social environment affects offspring sex ratio and has sex-specific effects on mortality, growth and

111   endocrine physiology. We expected overall positive effects on daughters of pair-housed females, i.e.

112   a bias towards female offspring because higher maternal androgen (Correa et al., 2011) and CORT

113   levels (Pike and Petrie, 2006) have been linked to a female-biased offspring sex ratio in Japanese

114   quail. Furthermore, we expected increased growth and decreased mortality in daughters of pair-

115   housed mothers because elevated maternal plasma androgen or CORT levels had positive effects on

116   daughters and negative effects on sons in other avian species (Love et al., 2005, Rutkowska and

117   Cichoń, 2006). In contrast to female-biased reproductive investment of pair-housed mothers, we

118   expected a potentially male-biased offspring sex ratio, increased growth and decreased mortality in

119   sons of group-housed mothers. We also investigated whether offspring from pair-housed and group-

120   housed mothers differ in their circulating androgen levels and the sensitivity of the HPA-axis.

121

2. Materials and methods

123   **2.1. Ethics statement**

124   All experimental procedures and humane endpoints for minimizing suffering were approved by the

125   North Rhine-Westphalia State Agency for Nature, Environment and Consumer Protection (Landesamt

126   für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen), Recklinghausen, Germany (licence

127   number 84-02.04.2013-A127). Animal facilities were approved for keeping and breeding Japanese

128 quail for research purposes by the local government authority responsible for health, veterinary and

129 food monitoring (Gesundheits-, Veterinär- und Lebensmittelüberwachungsamt Bielefeld, Germany).

130

131 **2.2. Parental generation**

132 The parental generation originated from eggs generously provided by the INRA in Nouzilly, France

133 (Experimental unit 1295 (UE PEAT) and UMR 85, Physiologie de la Reproduction et des

134 Comportements, INRA-CNRS-IFCE-Université de Tours, Val de Loire Center, Nouzilly, France). These

135 eggs were produced by females from a non-selected control line, bred next to quail lines selected for

136 low or high social reinstatement (Mills and Faure, 1991). They were incubated and reared at Bielefeld

137 University (Germany) and placed into their social treatments at 29 days of age. The social treatment

138 was either pair-housing or group-housing, with groups (n=12) consisting of three females and one

139 male, pairs (n=24) of one female and one male. Siblings or half siblings were never housed in the

140 same cage. The birds were kept indoors, in two adjacent rooms with artificial lighting and no natural

141 daylight. The light-dark cycle was 14:10h, and the temperature was set to 20°C. Pair cages measured

142 75 x 80 x 40 cm, group cages 150 x 80 x 40 cm. The distribution of the cages across and within rooms

143 was balanced across treatments. The birds were kept on wood shavings, and all cages contained a

144 sand bath and one shelter hut per female. Food (GoldDott Hennenmehl, Derby Spezialfutter GmbH,

145 Münster, Germany) and water was provided ad libitum. On a weekly basis, the standard diet was

146 supplemented with mealworms and shell grit. After collecting eggs for breeding the next generation,

147 the parental females were tested for their hormonal response to a stressor (at 66-67 days of age; see

148 Fig. 1) and to an injection with gonadotropin releasing hormone (at 72 days of age; see Fig. 1), and

149 we measured growth, reproductive output, and egg yolk testosterone concentrations (Langen et al.,

150 2017).

151

152 **2.3. Egg incubation and hatching**

153 After the parental generation had been housed in their treatment groups for 27 days (at 8 weeks of

154 age), eggs (124 eggs from pair-housed females and 155 eggs from group-housed females) were

155 collected over the course of one week, weighed to the nearest 0.1 g, and incubated to produce the

156 offspring generation. Eggs were incubated in a HEKA-Euro-Lux II incubator (HEKA-Brutgeräte,

157 Rietberg, Germany) in complete darkness to avoid the effects of light on development and because it

158 more likely reflects the situation during natural incubation (Archer and Mench 2014). Until

159 incubation day 14, the temperature was set at 37.8°C, humidity to 55%, and the eggs were turned

160 every 2 hours. After 9 days of incubation, the eggs were candled to identify embryonic development

161 and non-fertilized eggs were removed (remaining eggs: 107 eggs from pair-housed females and 121

162 eggs from group-housed females, Table 1). On day 15 of incubation, eggs were moved to hatching

163 trays, the incubation temperature was set to 37.5°C, the humidity to 75%, and the eggs were no

164 longer turned. The hatching trays were divided into separate compartments (5.5 x 5.5 x 5 cm) for

165 each individual egg so that we could identify which chick hatched from which egg. The compartment

166 walls were made of transparent Plexiglas and the bottom of each hatching tray was made of mesh

167 wire, allowing air flow and olfactory and acoustic communication between the chicks. The offspring

168 hatched after 17 ± 1 days of incubation. Some hatchlings were excluded from the experiment

169 because they had birth defects (two male offspring from two pair-housed mothers, one female

170 offspring from one group-housed mother). In addition, some offspring originated from cages in which

171 birds had to be separated before or during egg collection due to aggression (two male and one

172 female offspring from two mothers housed in the same group and six male and six female offspring

173 from two of five separated pairs; see Langen et al., 2017 for more information). These offspring from

174 separated parental cages were not included as subjects in the present study but used as cage mates.

175 A total of 35 male and 29 female offspring from pair-housed mothers, and 34 male and 33 female

176 offspring, and one hatchling of unidentified sex from group-housed mothers remained (Table 1).

177 Birds were removed from the incubator once their feathers had dried (ca. 2 hours after

178 hatching), weighed to the nearest 0.1 g and their tarsus was measured to the nearest mm using a

179 digital caliper. To measure circulating androgen levels at hatching and to assign genetic sex and

180 parentage, a small blood sample (max. 50 µl, or about 0.7% of body weight which does not appear to

181 have long-term effects on adult or developing birds; Sheldon et al. 2008) was taken by piercing the

182 jugular vein with a sterile 27 gauge needle and collecting the blood in heparinized capillaries (BRAND

183 GMBH + CO KG, Wertheim, Germany). As we were unable to retrieve blood from 25 out of 132

184 chicks, a piece of egg shell membrane (ca. 2 x 2 mm) containing blood vessels was collected for

185 genetic sex and parentage assignment. From the remaining 107 chicks, we were able to retrieve at

186 least a small amount of blood for DNA extraction, and 53 of these samples were further used for

187 androgen measurements (Table 1).

188   After 19 days of incubation, all eggs that had not hatched were removed from the incubator

189 and a tissue sample was taken from dead embryos for genetic sex determination.

190

191 **2.4. Offspring husbandry**

192 After weighing and measuring, the offspring were all kept together for the first night in a 100 x 80 x

193 80 cm cage on waved cardboard and with two heating lamps and food (ground pellets: GoldDott

194 Enten-Gänsestarter - no coccidiostat, Derby Spezialfutter GmbH, Münster, Germany) and water

195 provided ad libitum. Lights remained on for the first night. The next day, the birds were placed into

196 smaller groups of five to six unrelated individuals (all from the same parental treatment, n = 14 cages

197 of pair offspring, n = 12 cages of group offspring). At that time, the offsprings' sexes were still

198 unknown, therefore the chicks were randomly allocated across groups. Offspring cages measured 75

199 x 80 x 40 cm, contained heating pads partially covered by a small hut (15 x 13 x 13 cm), and ad

200 libitum water and food. The main lights were set to a 14:10h light-dark cycle (lights on at 5 am), but

201 small night lights were placed approximately 1 m in front of the cages to make sure the birds were

202 able to find food and water during the night. Birds were kept on waved cardboard until 8 days post-

203 hatching, after which they were kept on wood shavings.

204   All cages were checked daily, and we recorded whether any of the birds had died to be able

205   to measure differences in mortality between offspring from the two maternal treatments. To analyse

206   offspring growth, all birds were weighed to the nearest 0.1 g on the day of hatching, and on post-

207   hatching days 9 and 19. Between the day of hatching and day 23, the offspring underwent several

208   behavioural tests, the results of which will be described elsewhere (Langen et al. in prep). On post-

209   hatch day 20-21, we assessed the birds' CORT response to an injection with adrenocorticotropic

210   hormone. For a timeline of all experimental procedures, see Fig. 1. Sample sizes per measure vary

211   (Table 1) since some of the offspring died in the first few weeks or because we were unable to get

212   enough plasma for the physiological measurements.

213   **Table 1. Samples sizes for each measurement.**

Measure	Offspring from pair-housed mothers				Offspring from group-housed mothers			
	Total	sons	daughters	mothers	Total	sons	daughters	mothers (groups)
Sex ratio								
Primary	107[1]	49	47	18	121[1]	53	56	- [2] (8)
At hatching	64	35	29	17	68[1]	34	33	20 (8)
Correlation with maternal androgens	59	33	26	15	49	27	22	15 (6)
Correlation with maternal CORT	47	23	24	12	49[1]	25	23	16 (7)
At day 23	54	27	27	16	56	28	28	17 (7)
Mortality								
Overall	64	35	29	17	68[1]	34	33	20 (8)
Correlation with maternal androgens	59	33	26	15	49	27	22	15 (6)
Correlation with maternal CORT	47	23	24	12	49[1]	25	23	16 (7)
Egg mass	64	35	29	17	68[1]	34	33	20 (8)
Mass at hatching	64	35	29	17	68[1]	34	33	20 (8)
Mass at day 9	56	28	28	17	57	28	29	19 (8)
Mass at day 19	54	27	27	16	56	28	28	17 (7)
Hatchling androgens								
Individual samples	17	13	4	16	12	5	7	13 (7)
Pools	7	4 (8)[3]	3 (7)[3]	16	4	2 (4)[3]	2 (5)[3]	13 (7)
ACTH challenge								
Baseline	38[4]	22[4]	16[4]	15[4]	48[4]	26[4]	22[4]	16 (8)[4]
Response	37[4]	22[4]	15[4]	15[4]	47[4]	26[4]	21[4]	16 (8)[4]

214   [1] Sexing was unsuccessful in 23 embryos and 1 hatchling. [2] In groups, mothers of embryos were not identified. [3] Number of individuals included in the
215   plasma pool. [4] Reduced sample sizes due to insufficient plasma for the CORT analysis.

2.5. ACTH challenge

In order to test the offspring's HPA-axis sensitivity, we measured the plasma CORT increase following an injection of adrenocorticotropic hormone (ACTH, which stimulates glucocorticoid production in the adrenal glands and is normally released by the pituitary in response to corticotrophin-releasing hormone from the hypothalamus) on post-hatch day 20-21. All birds were tested between 09:00 am and 1:00 pm, and plasma CORT levels did not change significantly during that period ($\chi^2_{(1)}$: 1.55, p = 0.21).

For the ACTH challenge, all birds from one cage were caught and transported to the experimental room in a transport box (40 x 30 x 40 cm). A blood sample was taken to determine baseline plasma CORT concentrations by puncturing the ulnar vein with a sterile 27 gauge needle and collecting 200-300 µl blood in heparinised capillaries (BRAND GMBH + CO KG, Wertheim, Germany). We recorded the time between opening the cages and taking the baseline blood sample (range: 71-287 seconds, mean ± SEM: 155 ± 6 seconds).

After the baseline blood sample was taken, the birds were injected in the pectoral muscle with 0.8 µg ACTH (H-1150.0001, Bachem, Bubendorf, Switzerland) dissolved in 50 µl PBS (average dosage ca. 10 µg/kg) and placed back in the transport box. 10 minutes post injection, the birds were caught again, and a second blood sample was taken to determine the CORT response to ACTH.

2.6. Hormone analysis

After blood samples were taken to determine androgen (at the day of hatching) and CORT levels (in the ACTH challenge), samples were kept on ice for a maximum of two hours and then centrifuged for 10 minutes at 2000 x g. The plasma was then collected and frozen at -20°C for future use.

We used a commercial testosterone ELISA Kit (Demeditec Diagnostics GmbH, Kiel, Germany, cat. no. DES6622) to determine plasma androgen concentrations. Cross reactivity of the kit antibody, as reported by the manufacturer, was 23.3% for 5α-Dihydrotestosterone, 1.6% for Androstenedione, and less than 0.1% for other steroids. Samples were distributed over two assays, balanced for

maternal treatment. The inter-assay coefficient of variation (CV) was 1.64% (based on two quail plasma pools measured in each assay). Since we were unable to get sufficient plasma from 24 out of 53 chicks, the 24 samples were pooled by combining samples from two to three hatchlings in each pool, resulting in 11 plasma pools. We pooled plasma samples from hatchlings within the same sex and maternal treatment and, where possible, pools consisted of samples from full siblings. In total, 40 samples were measured in the T assay (29 single plasma samples and 11 plasma pools; Table 1). In four out of the 40 samples (two from sons of pair-housed mothers, two from daughters of group-housed mothers), androgen concentrations were below the range that could be estimated using the standard curve and were therefore assigned the lowest measured value (28.2 pg/ml), as a conservative estimate.

Plasma CORT concentrations in the ACTH challenge were determined using a commercial Corticosterone RIA Kit (MP Biomedicals, Orangeburg, USA, cat. no. 07-102102). Cross reactivity of the kit antibody, as reported by the manufacturer, was 0.34% for Desoxycorticosterone, 0.1% for Testosterone, and less than 0.1% for other steroids. Samples were measured together with quail plasma samples from other experiments and distributed over 11 assays, balanced for treatment. The intra-assay CV was 4.51%, the inter-assay CV was 13.86% (based on a chicken plasma pool and two kit controls measured in each assay). 170 samples were measured in the CORT assay (86 baseline, 84 post-ACTH; Table 1), and in 15 cases the CORT values were above the highest assay standard (all post-ACTH samples, from eight sons of pair-housed mothers and seven sons of group-housed mothers). As we were unable to repeat measurements at a higher dilution these samples were assigned a value of 35 ng/ml (based on the value of the highest assay standard) as a conservative estimate.

2.7. Genetic sex and parentage assignment

We used molecular methods to determine offspring sex and to assign parentage of all hatched offspring to one of the three potential mothers in the group treatment. The concentrated blood cells

268 left over after centrifuging blood for hormone measurements were diluted 1:2 with phosphate buffer

269 saline (10 mM PBS+6 mM EDTA, pH 7.4) and stored at -20°C. Similarly, tissue samples from non-

270 hatched embryos were frozen at -20°C for future use. Genomic DNA was obtained by a

271 phenol/chloroform or Chelex extraction (Walsh et al., 1991). Genetic sex determination was then

272 performed using primers 2550f and 2718r (Fridolfsson and Ellegren, 1999).

273 We genotyped offspring and parents at 22 microsatellite loci using fluorescently labelled

274 primers, as described previously (Langen et al., 2017). Parentage was then manually assigned by

275 identifying which genotype of the three potential mothers in a cage best matched the offspring

276 genotype.

277

278 **2.8. Statistics**

279 All statistical analyses were done using the lme4 package (Bates et al., 2015) of R 3.2.3 (R

280 Core Team, 2015).

281 To analyse the effect of the maternal social environment and maternal hormones on

282 offspring sex ratio and mortality, generalized linear mixed models with a binomial distribution and

283 logit link function were fitted. Models included the maternal social environment as a fixed effect.

284 Additionally, models of offspring mortality included a fixed effect of offspring sex and its interaction

285 with the maternal social environment. We tested for a sex-ratio bias in each of the maternal social

286 environments, where a significant effect of the intercept on the logit scale indicates a deviation from

287 parity. Finally, we tested for non-random (extra-binomial) variance of sex-ratios using simulations

288 (see Postma et al. 2011). We generated a distribution of 1000 expected clutch sex ratios based on

289 the observed mean sex ratio and the number of offspring from each mother or each maternal cage

290 (for embryos whose parentage was not assigned) and compared whether the observed variance in

291 sex-ratios fell outside the upper confidence interval (overdispersion) or lower confidence interval

292 (underdispersion) of the simulated data. We then analysed the effects of maternal hormones on

293 offspring sex ratio and mortality using separate models, either with maternal baseline plasma

294 androgen or with baseline plasma CORT levels as fixed effects. Maternal treatment was not included

295 in these models to avoid multicollinearity because maternal hormones differed according to

296 maternal treatment.

297 General linear mixed models were used to analyse the effect of the maternal social

298 environment and offspring sex on egg mass, offspring mass and offspring circulating hormone levels

299 (T at hatching and CORT during the ACTH challenge). Normality of the residuals from all general

300 linear mixed models was assessed visually using histograms and Q-Q plots. To achieve normality and

301 equal variances, we transformed values for offspring plasma CORT levels (square root) and body

302 mass (natural log). Again, fixed effects included the maternal social environment, offspring sex, and

303 their interactions. In addition, models of offspring growth included a categorical fixed effect of age

304 (in days) to model the increase in weight with age. The models also included all two-way and three-

305 way interactions of age with the maternal social environment and sex to test whether the weight

306 increase with age differed between treatments and sexes. Age was treated as a categorical fixed

307 effect because offspring mass was measured at only three time points (day 0, day 9 and day 19).

308 Models analysing effects on plasma CORT during the ACTH challenge included a fixed effect of

309 sample (pre or post-challenge) to test whether CORT increased in response to the challenge. The

310 models also included all two-way and three-way interactions of sample with the maternal social

311 environment and offspring sex to test whether the response to the challenge differed between

312 treatments and sexes. In addition, the models on plasma CORT included as a covariate the time it

313 took to collect the first sample after the initial disturbance of opening the cage.

314 Maternal cage was included as a random effect in all models, to control for potential non-

315 independence of mothers from the same cage. In addition, models included a random effect of

316 maternal ID nested within maternal cage, except for the models of primary sex ratio (because

317 parentage was only assigned for hatchlings, not for embryos). Models analysing offspring data

318 collected after the day of hatching also included a random effect of offspring cage. When analysing

319 offspring mortality, models did not converge if both maternal ID and offspring cage were includedas

320 random effects. Offspring cage was therefore removed from these models because it had a smaller

321 effect than maternal ID within maternal cage. For the analysis of growth and the ACTH challenge, we

322 included the additional random effects of offspring ID nested within offspring cage and offspring ID

323 nested within maternal ID nested within maternal cage.

324 We always started with the full model and stepwise excluded all non-significant (p > 0.05)

325 interactions and main effects, apart from the main factors of interest: the maternal social

326 environment and offspring sex. Interactions were always excluded before the main effects involved

327 in the interaction. We determined the significance of fixed effects using likelihood ratio tests

328 comparing the models with and without the factor of interest. The results of all models are reported

329 in the supplementary data.

330

331 3. Results

332 3.1. Offspring sex ratio and mortality

333 The maternal social environment did not affect primary sex ratio ($\chi^2_{(1)}$: 0.12, p = 0.73; Fig. 2), sex ratio

334 at hatching ($\chi^2_{(1)}$: 0.20, p = 0.65; Fig. 2) or sex ratio at day 23 ($\chi^2_{(1)}$ < 0.01, p = 0.99; Fig. 2). Sex ratios

335 did not differ significantly from parity at any stage in either social environment (all z values < 0.75, all

336 p values > 0.45; Supplementary Table 1), nor was there any evidence of over or underdispersion in

337 sex ratio at any stage (Supplementary Table 2). In addition, maternal baseline plasma hormone levels

338 did not predict offspring sex ratio at hatching (effect of maternal baseline androgens: $\chi^2_{(1)}$: 1.53, p =

339 0.22; effect of maternal baseline CORT: $\chi^2_{(1)}$: 0.36, p = 0.55; Supplementary Fig. 1).

340 The maternal social environment did not have sex-specific effects on offspring mortality

341 (effect of maternal social environment * offspring sex: $\chi^2_{(1)}$: 1.80, p = 0.18; Fig. 3), nor was there an

342 overall effect of the maternal social environment on mortality ($\chi^2_{(1)}$: 0.20, p = 0.66; Fig. 3). However,

343 mortality did differ between the sexes: significantly more male offspring than female offspring died

344 before day 23 ($\chi^2_{(1)}$: 4.48, p = 0.03; Fig. 3). Maternal baseline plasma hormone levels did not predict

345   offspring mortality (effect of maternal baseline androgens: $\chi^2_{(1)}$: 1.46, p = 0.23; effect of maternal

346   baseline CORT: $\chi^2_{(1)}$: 0.65, p = 0.42; Supplementary Fig. 1).

347

348   **3.2. Egg mass and growth**

349   The maternal social environment had no overall ($\chi^2_{(1)}$: 0.27, p = 0.60; Fig. 4A) or sex-specific effect on

350   egg weight (effect of maternal social environment * offspring sex: $\chi^2_{(1)}$: 0.02, p = 0.89; Fig. 4A), nor

351   did egg weight differ between the sexes ($\chi^2_{(1)}$: 0.25, p = 0.62; Fig. 4A).

352   All birds increased weight significantly over the course of the experiment (effect of age: $\chi^2_{(2)}$:

353   1531.30, p < 0.001; Fig. 4B). The changes in weight with age did not differ between the maternal

354   social environments (effect of maternal social environment * age: $\chi^2_{(2)}$: 0.49, p = 0.78; Fig. 4B) nor

355   between males and females (effect of offspring sex * age: $\chi^2_{(2)}$: 1.43, p = 0.49; Fig. 4B) or depending

356   upon the interaction between maternal social environment and offspring sex (effect of maternal

357   social environment * age * offspring sex: $\chi^2_{(2)}$: 4.63, p = 0.10; Fig. 4B). There was no difference in

358   average offspring mass according to the maternal social environment, offspring sex, or their

359   interaction (the model included the significant effect of age; all $\chi^2_{(1)}$ < 1.64, all p values > 0.20).

360

361   **3.3. Offspring physiology**

362   The maternal social environment had no sex-specific effects on offspring plasma androgen

363   concentrations at hatching (effect of maternal social environment * offspring sex: $\chi^2_{(1)}$: 0.02, p = 0.89;

364   Fig. 5A). Average androgen concentrations also did not differ between offspring of pair-housed and

365   group-housed mothers ($\chi^2_{(1)}$: 0.45, p = 0.50; Fig. 5A), nor between males and females ($\chi^2_{(1)}$: 1.92, p =

366   0.17; Fig. 5A).

367   The maternal social environment did not affect the CORT response to an injection with ACTH

368   on post hatch day 20-21 (effect of maternal social environment * sample: $\chi^2_{(1)}$: 0.58, p = 0.45; Fig. 5B).

369   Male and female offspring differed in their CORT response (effect of offspring sex * sample: $\chi^2_{(1)}$:

370   7.11, p < 0.01; Fig. 5B) but the sex difference in the CORT response was not affected by the maternal

371 social environment (effect of sample * maternal social environment * offspring sex: $\chi^2_{(1)}$: 2.62, p =

372 0.11). The time between the initial disturbance of opening the cage and the collection of the baseline

373 sample was included as a covariate in all models analysing the effects on ACTH because it

374 significantly affected CORT levels ($\chi^2_{(1)}$: 8.34, p < 0.01). This effect did not differ between offspring

375 from the different maternal social environments ($\chi^2_{(1)}$: 0.19, p = 0.66). Removing the factor "time

376 until the first sample" from these models did not change the significance or interpretation of the

377 main effects. When analysing CORT baseline and response levels separately, male and female

378 offspring did not differ in baseline CORT concentrations ($\chi^2_{(1)}$ = 0.02, p = 0.89; Fig. 5B), but males had

379 significantly higher CORT concentrations after the ACTH injection ($\chi^2_{(1)}$: 16.33, p < 0.001; Fig. 5B).

380 CORT concentrations increased significantly in both sexes after the ACTH injection (males: $\chi^2_{(1)}$:

381 146.79, p < 0.001; females: $\chi^2_{(1)}$: 86.44, p < 0.001; Fig. 5B).

382

4. Discussion

384 The social environment a female is exposed to during reproduction has been reported to induce

385 variation in offspring sex-ratio, mortality, development, and endocrine physiology in a number of

386 vertebrate species (Dantzer et al., 2013; Guibert et al., 2010; Kaiser and Sachser, 2009, 2005; Michler

387 et al., 2013; Minias et al., 2014; von Engelhardt et al., 2015). Maternal hormones are candidate

388 signals involved in such transgenerational effects (Dantzer et al., 2013; Guibert et al., 2010; Hayward

389 and Wingfield, 2004; Henriksen et al., 2013), and they are thought to represent important proximate

390 mechanisms in adaptive sex allocation (Navara, 2013a, 2013b), also by affecting secondary offspring

391 sex ratios (Love et al., 2005; Rutkowska et al., 2007; Rutkowska and Cichoń, 2006).

392 We did not find evidence that the maternal social environment (pair versus group housing)

393 affects offspring sex ratio in Japanese quail, even though pair-housed females had increased

394 circulating androgen levels and a non-significant trend of higher cortisol levels compared to group-

395 housed females, as reported in our previous study (Langen et al., 2017). We had predicted that pair-

396 housed females would produce a female-biased offspring sex-ratio because increased androgen and

17

397 CORT levels were associated with a female-biased sex-ratio in other studies on Japanese quail

398 (Correa et al., 2011, Pike and Petrie, 2006). Offspring from pair-housed mothers and offspring from

399 group-housed mothers also did not differ in growth, mortality, circulating androgen levels or

400 circulating CORT levels. Moreover, maternal circulating levels of androgens and CORT did not

401 correlate with offspring sex ratio and mortality.

402   Our results contradict the general pattern in avian species which suggests that higher

403 maternal androgens lead to a male-biased offspring sex ratio (Goerlich et al., 2009; Navara, 2013a,

404 2013b), but we corroborate earlier findings in Japanese quail showing no such relationship (Pike and

405 Petrie, 2006). However, in Japanese quail, higher maternal androgens have also been linked to

406 female-biased sex ratios (Correa et al., 2011), indicating that the effect of maternal androgens on

407 offspring sex ratio is still unclear. Our results also do not confirm earlier reports that higher maternal

408 CORT levels are linked to a female-biased offspring sex ratio in avian species (Navara, 2013a, 2013b),

409 including Japanese quail (Pike and Petrie, 2006). Studies investigating the relationship between

410 maternal plasma hormone levels and offspring sex ratio differ substantially regarding methods of

411 hormone manipulation or quantification which might explain differing results between studies. For

412 example, Correa et al. (2011) found the temporary peak in circulating androgen levels following

413 mating to be correlated with a female-biased sex ratio. On the other hand, Pike and Petrie (2006),

414 who found no relationship between offspring sex ratio and androgens, analysed faecal androgen

415 metabolite concentrations, which do not reflect short-term fluctuations but an integrated measure

416 of androgen concentrations over several hours. They also found no effect of treating females with

417 androgen implants, which likely affected circulating androgen levels over a longer time period. In

418 addition, multiple steroid hormones are thought to be involved in sex ratio adjustment, and their

419 effects may interact (Navara, 2013a). In the present study, the opposing effects of higher maternal

420 androgens and higher CORT on offspring sex ratio may have cancelled each other out, explaining why

421 the offspring sex ratio of pair-housed mothers did not differ from parity nor from that of group-

422 housed females. Moreover, the elevation in maternal plasma androgen and CORT levels in pair-

423   housed females may not have been large enough to induce a shift in offspring sex ratio. Finally, it has

424   been suggested that effects on primary sex ratios may be largely due to variation in levels of

425   progesterone during meiosis, which is the main follicular steroid produced during this phase (Correa

426   et al. 2005) but was not measured in our study.

427   The lack of an effect of the maternal social environment on offspring growth, mortality or

428   physiology might partly be explained by the fact that the maternal social environment did not induce

429   differences in yolk androgens, as shown in our previous study (Langen et al., 2017), or in egg mass, as

430   shown here. Yolk hormones are considered a key mechanism in transferring the effects of the

431   maternal social environment to offspring (Gil, 2008; Rutkowska and Cichoń, 2006; von Engelhardt et

432   al., 2006; von Engelhardt and Groothuis, 2011), and differences in the maternal social environment

433   and physiology alone may not suffice to induce changes in the prenatal environment. The fact that

434   we found no effects on egg mass can also explain why offspring growth and mortality did not differ,

435   egg size being another important mediator of maternal effects (Cunningham & Russell 2000; Hadfield

436   et al. 2013; Krist, 2011; Pick et al. 2016; Williams, 1994). We also found no sex differences in egg

437   mass, confirming previous suggestions that there is little evidence overall for sexual size dimorphism

438   in eggs across avian species (Rutkowska et al. 2014).

439   We did find a difference in the physiological stress response (increase in CORT) after an ACTH

440   injection between male and female offspring, irrespective of the maternal social environment. While

441   baseline CORT concentrations did not differ between males and females, males showed a higher

442   CORT response, suggesting increased sensitivity of their HPA axis. This is in line with many studies in

443   birds, including Japanese quail, that report a stronger stress response in males compared to females

444   (Astheimer et al., 1994; Goerlich et al., 2012; Hayward et al., 2006; Hazard et al., 2008; Krause et al.,

445   2015; Madison et al., 2008; Romero et al., 2006; Schmeling and Nockels, 1978). Other studies,

446   however, report no differences (Dufty Jr. and Belthoff, 1997; Hazard et al., 2008; Satterlee and

447   Johnson, 1988; Sockman and Schwabl, 2001). In addition to having a higher stress response,

448   significantly more male offspring died before day 23 than female offspring. Similar patterns in

449   juvenile mortality are reported in a large number of species (reviewed by Clutton-Brock, 1991),

450   suggesting that males are more vulnerable to environmental challenges. However, it is still unclear

451   what the underlying mechanisms are (Jones et al., 2009).

452   Overall, contradictory findings regarding the effects of maternal physiology and maternal

453   social environment on offspring sex ratio and phenotypes indicate that the mechanisms underlying

454   such maternal effects are still insufficiently understood. An important factor explaining differences

455   between studies, including our own and previous research, might be the timing of manipulations and

456   measurements of the social environment and the endocrine system. Effects of the maternal

457   environment and physiology on developing follicles and offspring may occur only during critical

458   windows (Okuliarova et al., 2017). For example, for sex ratio adjustment, an influence of maternal

459   steroids on the segregation of the sex chromosomes during the second meiotic division has been

460   proposed (Correa et al., 2005; Goerlich-Jansson et al., 2013; Navara, 2013a, 2013b; Pinson et al.,

461   2011; Rutkowska and Badyaev, 2008). Also, circulating hormone levels differ between life stages and

462   seasons and can change significantly during a single day, even within minutes, in response to the

463   environment, such as social stimuli (Adkins-Regan, 2005; Creel et al., 2013; Hazard et al., 2005;

464   Oliveira, 2004; Ottinger et al., 2001). A single measurement of physiological status does not take such

465   fluctuations into account and might reduce the chance of detecting maternal effects. We may have

466   also missed important effects by not measuring maternal hormones during the time window during

467   which genetic sex determination takes place (meiosis I) and by only measuring maternal androgens

468   and corticosterone, not other steroids such as progesterone (Correa et al., 2005).

469   Finally, differences between the social stimuli investigated may explain the contradictory

470   results between studies. Social factors such as maternal social instability (Guibert et al., 2010; Kaiser

471   and Sachser, 2009), social density (Dantzer et al., 2013; Minias et al., 2014; von Engelhardt et al.,

472   2015), mate attractiveness (Kölliker et al., 1999; Korsten et al., 2006; Rutstein et al., 2005; Sheldon et

473   al., 1999; Svensson and Nilsson, 1996), pair bonding (Hirschenhauser, 2012; Le Bot et al., 2014;

474   Schweitzer et al., 2014), and social status (Dloniak et al., 2006) are likely to differ in their functional

475 significance and therefore also in their effects on offspring phenotypes and sex ratio. To gain a better

476 understanding of the underlying mechanisms and the function of maternal effects of the social

477 environment, it is therefore necessary to establish which social stimuli are most important for

478 offspring, and at which time maternal effects manifest in relation to the prenatal and postnatal

479 developmental stages.

480

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491

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Figure and supplementary captions

734   **Fig. 1.** Timeline of experimental procedures. Procedures marked with * are behavioural tests which

735   are not reported here.

736

737   **Fig. 2.** Offspring sex ratio at the embryonic stage, at hatching and at day 23. Data shown are the

738   estimated means ± 1 SEM (back-transformed from logit).

739

740   **Fig. 3.** The proportion of offspring that died before day 23. Data shown are the estimated means ± 1

741   SEM (back-transformed from logit).

742

743   **Fig. 4.** A: egg mass. B: offspring growth (back-transformed from natural log). Data shown are the

744   estimated means ± 1 SEM.

745

746   **Fig. 5.** A: offspring plasma androgen concentrations (pg/ml) at hatching. B: offspring plasma CORT

747   concentrations (ng/ml) before and 10 minutes after the ACTH injection (back-transformed from

748   square root). Data shown are the estimated means ± 1 SEM.

749

750   **Supplementary Fig. 1.** Relationship between maternal hormones and offspring sex ratio at hatching

751   and mortality. A: maternal androgens and offspring sex ratio at hatching, B: maternal CORT and

752   offspring sex ratio at hatching, C: maternal androgens and offspring mortality, D: maternal CORT and

753   offspring mortality.

754

755   **Supplementary Data 1.** Raw dataset.

756

757   **Supplementary Data 2.** Supplementay tables, including summaries of all model outputs.
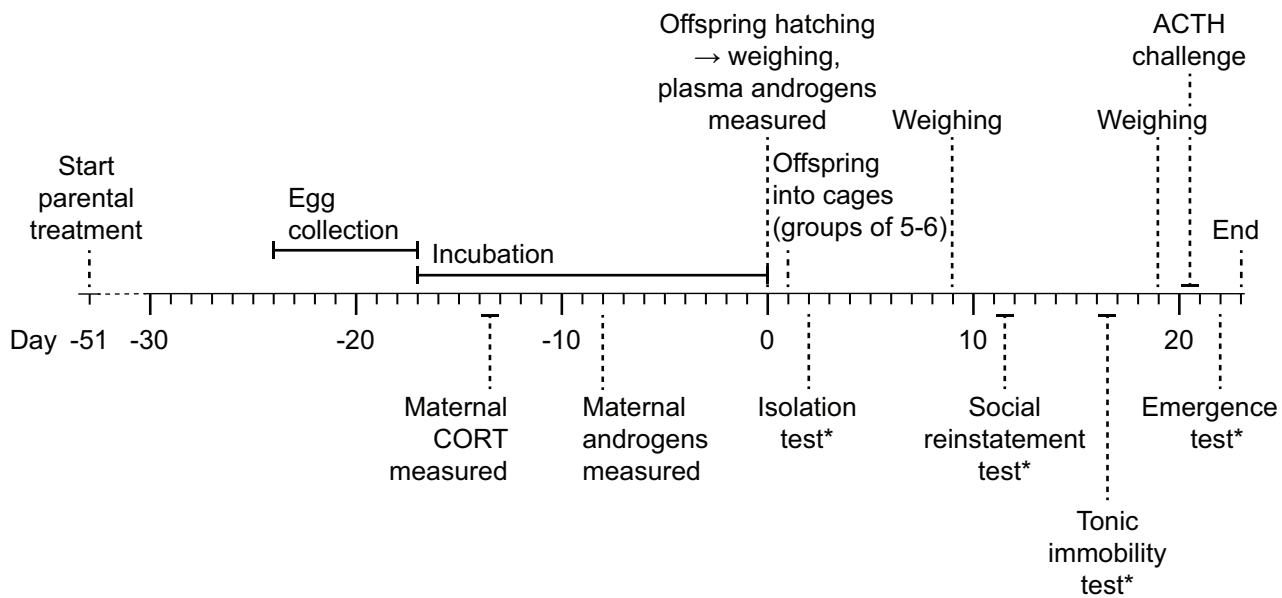
Supplementary table 1. Sex ratio deviations from parity at the embryonic stage (primary sex ratio), at hatching and at day 23.

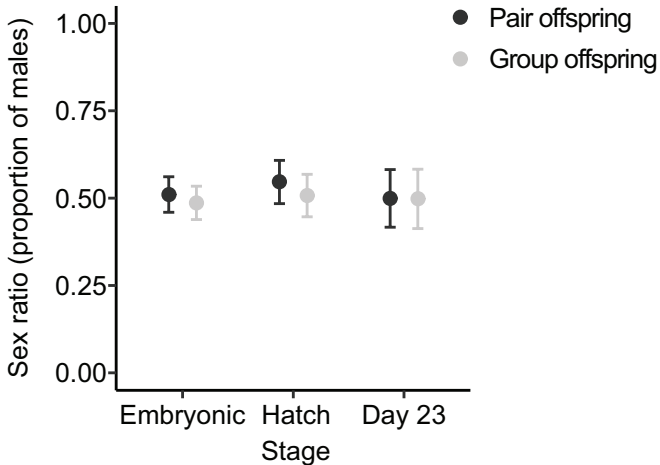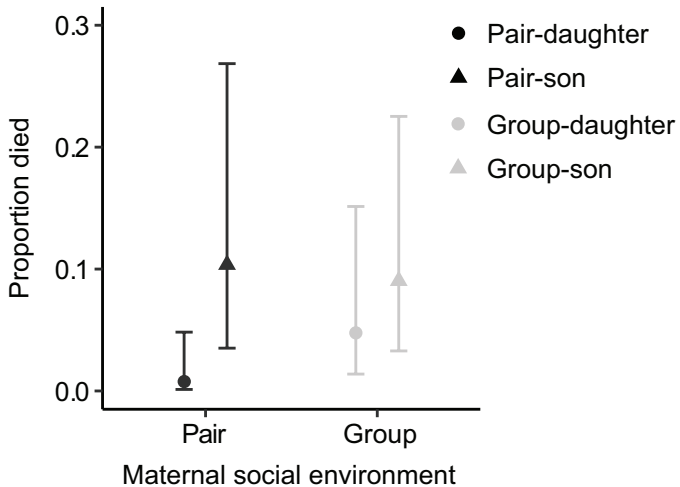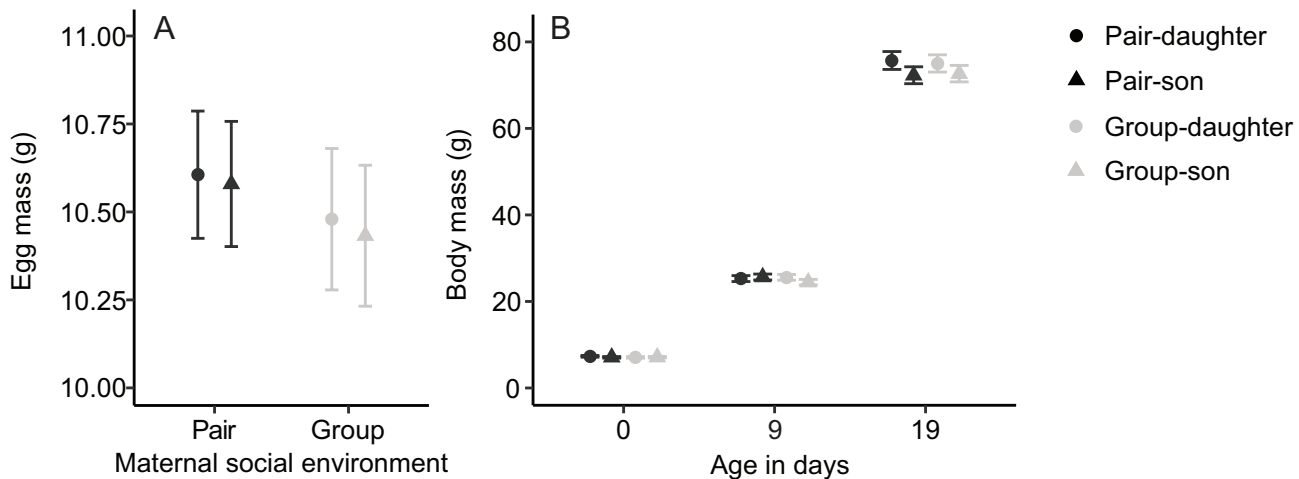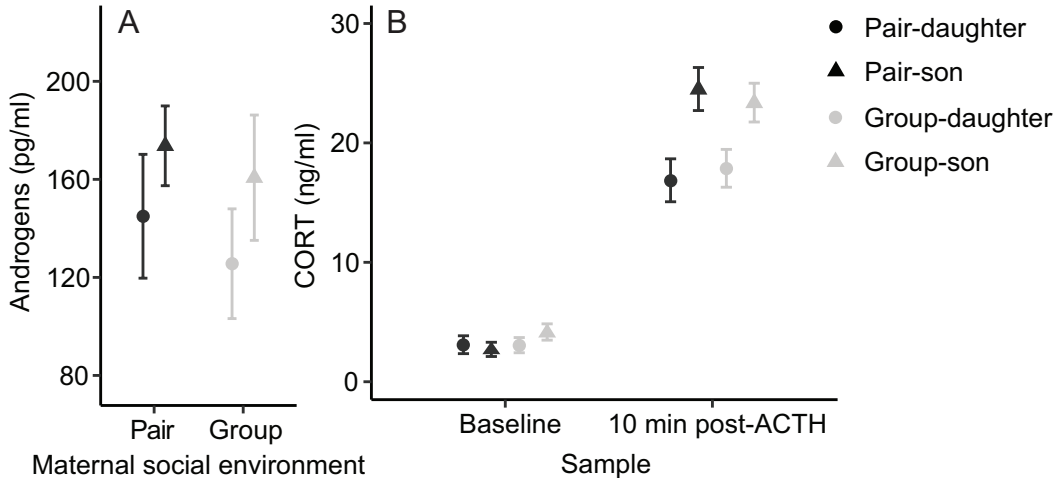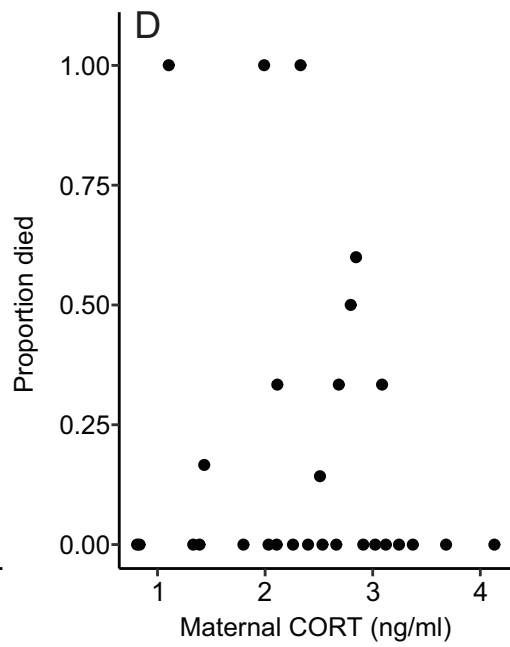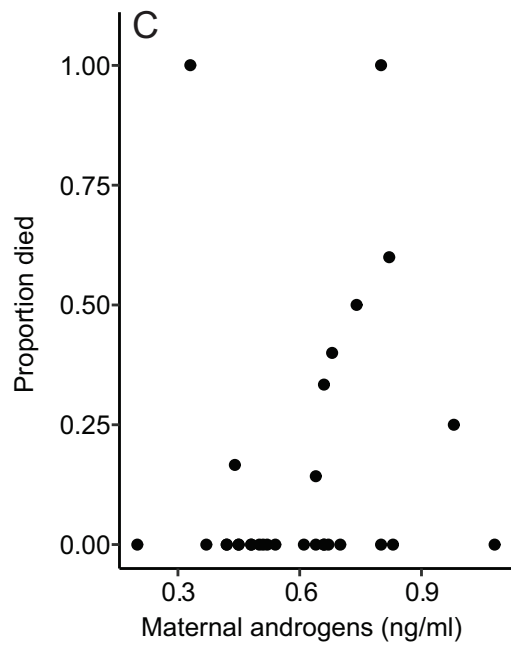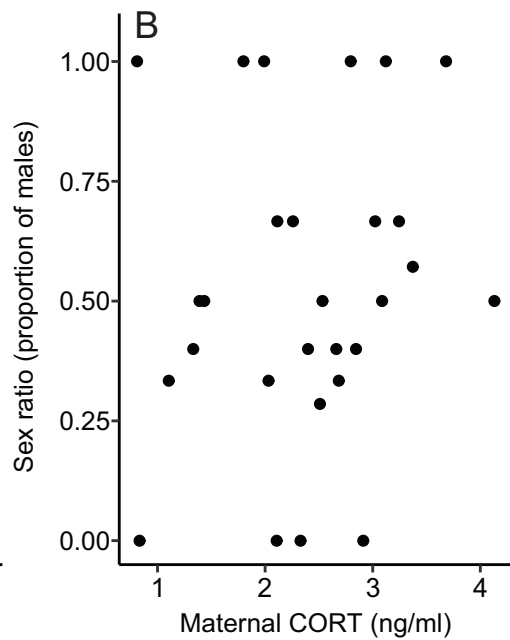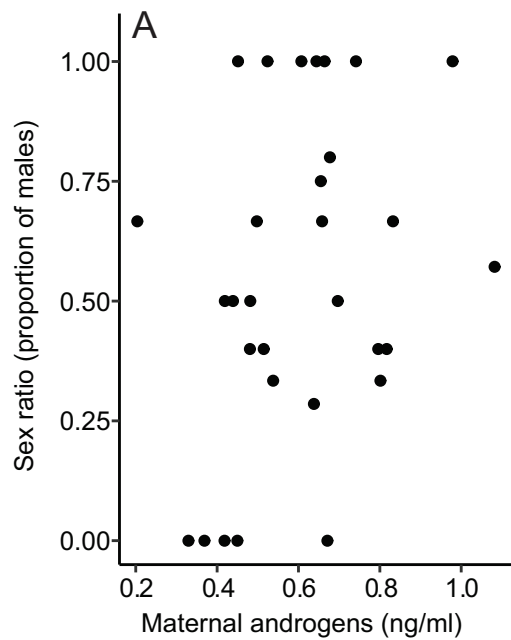|	Pair-housed mothers			Group-housed mothers		
Stage	Intercept ± SE	z	p	Intercept ± SE	z	p
Primary	0.04 ± 0.20	0.20	0.84	-0.06 ± 0.19	-0.29	0.77
Hatch	0.19 ± 0.25	0.75	0.45	0.03 ± 0.24	0.12	0.90
Day 23	0.003 ± 0.29	0.01	0.99	0.00 ± 0.27	0.00	1.00

Supplementary table 2. Observed and expected variance in sex ratios at the embryonic stage (primary sex ratio), at hatching and at day 23.

Stage	Observed variance	Median expected variance (95% CI)
Primary	0.05	0.04 (0.02 – 0.07)
Hatch	0.10	0.10 (0.08 – 0.13)
Day 23	0.12	0.10 (0.08 – 0.14)

Supplementary table 3. Generalized linear mixed model of effects of the maternal social environment on primary sex ratio.

Fixed effects:					
	Estimate	SE	χ^2	df	p
Intercept	0.042	0.204			
Maternal social environment	-0.097	0.280	0.12	1	0.73
Random effects:					
	Variance	Std.Dev.			
Maternal cage	0[a]	0[a]			

Estimates are given on the logit scale. Maternal pair-housing is coded as 0, maternal group-housing is coded as 1. Maternal ID is not included because parentage was not assigned for embryos.

[a] variance parameters estimated as zero in the model

Supplementary table 4. Generalized linear mixed model of effects of the maternal social environment on sex ratio at hatching.

Fixed effects:					
	Estimate	SE	χ^2	df	p
(Intercept)	0.188	0.251			
Maternal social environment	-0.158	0.350	0.20	1	0.65
Random effects:					
	Variance	Std.Dev.			
Maternal cage : maternal ID[a]	0[b]	0[b]			
Maternal cage	0[b]	0[b]			

Estimates are given on the logit scale. Maternal pair-housing is coded as 0, maternal group-housing is coded as 1.

[a] maternal ID nested within maternal cage

[b] variance parameters estimated as zero in the model

Supplementary table 5. Generalized linear mixed model of effects of the maternal social environment on sex ratio at day 23.

Fixed effects:					
	Estimate	SE	χ^2	df	p
(Intercept)	-0.003	0.333			
Maternal social environment	-0.006	0.478	< 0.01	1	0.99
Random effects:					
	Variance	Std.Dev.			
Maternal cage : maternal ID[a]	$1.33*10^{-10}$	$1.15*10^{-5}$			
Maternal cage	0.024	0.156			
Offspring cage	0.344	0.587			

Estimates are given on the logit scale. Maternal pair-housing is coded as 0, maternal group-housing is coded as 1.

[a] maternal ID nested within maternal cage

Supplementary table 6. Generalized linear mixed model of effects of maternal baseline plasma androgens on sex ratio at hatching

Fixed effects:					
	Estimate	SE	χ^2	df	p
(Intercept)	-0.551	0.661			
Maternal androgens (ng/ml)	1.228	1.007	1.53	1	0.22
Random effects:					
	Variance	Std.Dev.			
Maternal cage : maternal ID[a]	0[b]	0[b]			
Maternal cage	0[b]	0[b]			

Estimates are given on the logit scale.

[a] maternal ID nested within maternal cage

[b] variance parameters estimated as zero in the model

Supplementary table 7. Generalized linear mixed model of effects of maternal baseline plasma CORT on sex ratio at hatching

Fixed effects:					
	Estimate	SE	χ^2	df	p
(Intercept)	-0.349	0.650			
Maternal CORT (ng/ml)	0.155	0.259	0.36	1	0.55
Random effects:					
	Variance	Std.Dev.			
Maternal cage : maternal ID[a]	0[b]	0[b]			
Maternal cage	0[b]	0[b]			

Estimates are given on the logit scale.

[a] maternal ID nested within maternal cage

[b] variance parameters estimated as zero in the model

Supplementary table 8. Generalized linear mixed model of effects of the maternal social environment on offspring mortality

Fixed effects:

	Estimate	SE	χ^2	df	p
(Intercept)	-3.887	1.444			
Offspring sex * maternal social environment[a]	-1.994	1.579	1.80	1	0.18
Offspring sex	1.494	0.808	4.48	1	0.03
Maternal social environment	0.487	1.119	0.20	1	0.66

Random effects:

	Variance	Std.Dev.			
Maternal cage : maternal ID[b]	5.752	2.398			
Maternal cage	$4.26*10^{-10}$	$2.06*10^{-5}$			

Estimates are given on the logit scale. Maternal pair-housing is coded as 0, maternal group-housing is coded as 1. Female offspring are coded as 0, male offspring are coded as 1.

[a] estimates and statistics are from the last model that still included the interaction.

[b] maternal ID nested within maternal cage

Supplementary table 9. Generalized linear mixed model of effects of maternal baseline plasma androgens on offspring mortality

Fixed effects:

	Estimate	SE	χ^2	df	p
(Intercept)	-5.162	2.252			
Maternal androgens (ng/ml)	3.517	2.955	1.46	1	0.23

Random effects:

	Variance	Std.Dev.			
Maternal cage : maternal ID[a]	3.708	1.926			
Maternal cage	$3.27*10^{-10}$	$1.81*10^{-5}$			

Estimates are given on the logit scale.

[a] maternal ID nested within maternal cage

Supplementary table 10. Generalized linear mixed model of effects of maternal baseline plasma CORT on offspring mortality

Fixed effects:

	Estimate	SE	χ^2	df	p
(Intercept)	-1.224	1.710			
Maternal CORT (ng/ml)	-0.549	0.690	0.65	1	0.42

Random effects:

	Variance	Std.Dev.			
Maternal cage : maternal ID[a]	2.780	1.668			
Maternal cage	0.522	0.722			

Estimates are given on the logit scale.

[a] maternal ID nested within maternal cage

Supplementary table 11. General linear mixed model of effects of the maternal social environment on egg mass

Fixed effects					
	Estimate	SE	χ^2	df	p
(Intercept)	10.612	0.176			
Offspring sex * Maternal social environment[a]	-0.021	0.148	0.02	1	0.89
Offspring sex	-0.037	0.074	0.25	1	0.62
Maternal social environment	-0.137	0.259	0.27	1	0.60
Random effects					
	Variance	Std.Dev.			
Maternal cage : maternal ID[b]	0.295	0.543			
Maternal cage	0.156	0.395			
Residual	0.138	0.371			

Estimates are given on the original scale. Maternal pair-housing is coded as 0, maternal group-housing is coded as 1. Female offspring are coded as 0, male offspring are coded as 1.

[a] estimates and statistics are from the last model that still included the interaction.

[b] maternal ID nested within maternal cage

Supplementary table 12. General linear mixed model of effects of the maternal social environment on offspring growth

Fixed effects

	Estimate	SE	χ^2	df	p
(Intercept)	1.983	0.022			
Day9 * Maternal social environment * Offspring sex[a]	-0.098	0.047	4.63	2	0.10
Day19 * Maternal social environment * Offspring sex[a]	-0.025	0.047			
Maternal social environment * Offspring sex[a]	0.001	0.033	< 0.01	1	0.97
Day9 * Maternal social environment[a]	-0.003	0.024	0.49	2	0.78
Day19 * Maternal social environment[a]	0.013	0.024			
Day9 * Offspring sex[a]	-0.003	0.024	1.43	2	0.49
Day19 * Offspring sex[a]	-0.026	0.024			
Day9	1.262	0.012	1531.30	2	< 0.001
Day19	2.336	0.012			
Maternal social environment	-0.011	0.027	0.17	1	0.68
Offspring sex	-0.021	0.016	1.64	1	0.20

Random effects

	Variance	Std.Dev.
Maternal cage : maternal ID : F1 ID[b]	0.002	0.045
Maternal cage : maternal ID[c]	0.004	0.065
Maternal cage	0[e]	0[e]
Offspring cage : F1 ID[d]	0.002	0.045
Offspring cage	$5.03*10^{-19}$	$7.09*10^{-10}$
Residual	0.008	0.091

Estimates are given on the natural log scale. Maternal pair-housing is coded as 0, maternal group-housing is coded as 1. Female offspring are coded as 0, male offspring are coded as 1. Effects of Day 9 and Day 19 are estimated in relation to Day 0 (hatching).

[a] estimates and statistics are from the last model that still included the interaction.

[b] offspring ID nested within maternal ID nested within maternal cage

[c] maternal ID nested within maternal cage

[d] offspring ID nested within offspring cage

[e] variance parameters estimated as zero in the model

Supplementary table 13. General linear mixed model of effects of the maternal social environment on offspring plasma androgens at hatching.

Fixed effects					
	Estimate	SE	χ2	df	p
(Intercept)	143.092	20.962			
Maternal social environment * Offspring sex[a]	6.354	44.912	0.02	1	0.89
Maternal social environment	-16.253	22.925	0.45	1	0.50
Offspring sex	31.390	22.280	1.92	1	0.17
Random effects					
	Variance	Std.Dev.			
Maternal cage : maternal ID (or plasma pool)[b]	8.64^*10^{-13}	9.30^*10^{-7}			
Maternal cage	135.553	11.643			
Residual	4338.397	65.867			

Estimates are given on the original scale. Maternal pair-housing is coded as 0, maternal group-housing is coded as 1. Female offspring are coded as 0, male offspring are coded as 1.

[a] estimates and statistics are from the last model that still included the interaction.

[b] maternal ID (or plasma pool) nested within maternal cage

Supplementary table 14. General linear mixed model of effects of the maternal social environment on offspring CORT in the ACTH challenge

Fixed effects

	Estimate	SE	χ2	df	p
(Intercept)	1.204	0.226			
Time of day[a]	-1.594	1.269	1.49	1	0.22
Sample * Maternal social environment * Offspring sex[a]	-0.787	0.483	2.62	1	0.11
Maternal social environment * Offspring sex[a]	-0.058	0.263	0.05	1	0.83
Maternal social environment * time until first sample [a]	0.001	0.002	0.19	1	0.66
Sample * Maternal social environment[a]	-0.184	0.242	0.58	1	0.45
Sample	2.379	0.183			
Maternal social environment	0.088	0.153	0.33	1	0.57
Offspring sex	0.065	0.176			
Time until first sample	0.003	0.001	8.34	1	< 0.01
Sample * Offspring sex	0.654	0.242	7.11	1	< 0.01

Random effects

	Variance	Std.Dev.
Maternal cage : maternal ID : F1 ID[b]	0[e]	0[e]
Maternal cage : maternal ID[c]	0.056	0.236
Maternal cage	0[e]	0[e]
Offspring cage : F1 ID[d]	0[e]	0[e]
Offspring cage	0[e]	0[e]
Residual	0.603	0.777

Split by sample:

Baseline

Fixed effects

	Estimate	SE	χ2	df	p
(Intercept)	0.600	0.150			
Maternal social environment	0.132	0.107	1.47	1	0.23
Offspring sex	-0.015	0.095	0.02	1	0.89
Time until first sample	0.007	0.001	54.54	1	< 0.001

Random effects

	Variance	Std.Dev.
Maternal cage : maternal ID[c]	0.014	0.118
Maternal cage	0[e]	0[e]
Offspring cage	0.005	0.069
Residual	0.169	0.411

Response

Fixed effects

	Estimate	SE	χ2	df	p
(Intercept)	4.049	0.355			
Maternal social environment	$2.88*10^{-5}$	0.348	< 0.01	1	0.99
Offspring sex	0.912	0.210	16.33	1	< 0.001
Time until first sample	$-3.19*10^{-4}$	0.002	0.03	1	0.86

Random effects	Variance	Std.Dev.
Maternal cage : maternal ID[c]	0[e]	0[e]
Maternal cage	0.362	0.601
Offspring cage	0.043	0.206
Residual	0.674	0.821

Split by sex:

Males

Fixed effects	Estimate	SE	χ2	df	p
(Intercept)	1.390	0.269			
Sample	3.034	0.155	146.79	1	< 0.001
Maternal social environment	0.059	0.192	0.10	1	0.76
Time until first sample	0.003	0.001	3.25	1	0.07

Random effects	Variance	Std.Dev.
Maternal cage : maternal ID : F1 ID[b]	0[e]	0[e]
Maternal cage : maternal ID[c]	0.069	0.263
Maternal cage	0[e]	0[e]
Offspring cage : F1 ID[d]	$2.60*10^{-15}$	$5.10*10^{-8}$
Offspring cage	$5.75*10^{-16}$	$2.40*10^{-8}$
Residual	0.580	0.761

Females

Fixed effects	Estimate	SE	χ2	df	p
(Intercept)	0.995	0.330			
Sample	2.385	0.169	86.44	1	< 0.001
Maternal social environment	0.119	0.282	0.18	1	0.68
Time until first sample	0.005	0.002	6.41	1	0.011

Random effects	Variance	Std.Dev.
Maternal cage : maternal ID : F1 ID[b]	0[e]	0[e]
Maternal cage : maternal ID[c]	0[e]	0[e]
Maternal cage	0.182	0.427
Offspring cage : F1 ID[d]	0[e]	0[e]
Offspring cage	0[e]	0[e]
Residual	0.513	0.716

Estimates are given on the square root scale. Maternal pair-housing is coded as 0, maternal group-housing is coded as 1. Female offspring are coded as 0, male offspring are coded as 1. The baseline sample in the ACTH challenge is coded as 1, the post-ACTH sample is coded as 2.

[a] estimates and statistics are from the last model that still included the variable or interaction.

[b] offspring ID nested within maternal ID nested within maternal cage

[c] maternal ID nested within maternal cage

[d] offspring ID nested within offspring cage

[e] variance parameters estimated as zero in the model