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RESEARCH ARTICLE

Effects of the maternal and current social environment on female body mass and reproductive traits in Japanese quail (*Coturnix japonica*)

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ABSTRACT

The social environment of breeding females can affect their phenotype, with potential adaptive maternal effects on offspring that experience a similar environment. We housed Japanese quail (*Coturnix japonica*) females in two group sizes (pairs versus groups of four) and studied the effects on their offspring under matched and mismatched conditions. We measured F1 body mass, reproduction, and plasma levels of androgens and corticosterone. F1 group housing led to an increase in body mass. In addition, F1 group housing had a positive effect on mass in daughters of pair-housed P0 females only, which were heaviest under mismatched conditions. At the time of egg collection for the F2 generation, F1 group-housed females were heavier, irrespective of the P0 treatment. F1 females in groups laid heavier eggs, with higher hatching success, and produced heavier offspring, most likely a maternal effect of F1 mass. F1 plasma hormones were affected by neither the P0 nor the F1 social environment. These results contrasted with effects in the P0 generation (reported previously), in which plasma hormone levels, but not mass, differed between social environments. This may be due to changes in adult sex ratios as P0 females were housed with males, whereas F1 females encountered males only during mating. Our study demonstrates potentially relevant mismatch effects of the social environment on F1 body mass and maternal effects on F2 offspring, but further study is needed to understand their adaptive significance and physiological mechanisms.

KEY WORDS: Transgenerational effects, Group size, Reproductive investment, Steroid hormones, Physiology, Morphology

INTRODUCTION

Effects of the maternal social environment on female physiology, reproduction and offspring phenotype have been described in various species, including birds and mammals (Groothuis et al., 2005; Guibert et al., 2010; Kaiser and Sachser, 2005, 2009). Maternal effects can act as mechanisms of adaptive transgenerational plasticity to optimally prepare offspring phenotype for their future environment. This can be tested by studying the consequences for offspring experiencing an environment that matches or mismatches the maternal environment (Burgess and Marshall, 2014; Marshall and Uller, 2007; Uller et al., 2013). This study investigated the transgenerational effects of maternal social group size on offspring housed under either matched or mismatched social conditions in an avian species, the Japanese quail (*Coturnix japonica* Temminck and Schlegel 1849).

Behaviour, physiology and reproduction can be affected by properties of the social environment, such as population density, group size, social rank, mate attractiveness or adult/operational sex ratio (Alonso-Alvarez et al., 2012; Asghar Saki et al., 2012; Benyi et al., 2006; Both, 1998; Both et al., 2000; Clutton-Brock and Huchard, 2013; Cunningham and Russell, 2000; Dewsbury, 1982; Ellis, 1995; Fowler, 1981; Rodenhouse et al., 2003; Schubert et al., 2007; Sillett et al., 2004; Stockley and Bro-Jørgensen, 2011; Székely et al., 2014; Uller et al., 2005). Effects of the social environment on female endocrine physiology and body mass (Bonenfant et al., 2009; DeVries et al., 2003; Eisenegger et al., 2011) provide proximate mechanisms through which reproduction and offspring can be affected. In birds, increasing group size, for example, is thought to exacerbate intraspecific competition, which can affect body mass (Asghar Saki et al., 2012; Keeling et al., 2003; Onbaşılar and Aksoy, 2005) and circulating levels of steroid hormones such as corticosterone and androgens (Cantarero et al., 2015; Cunningham et al., 1987; Koelkebeck and Cain, 1984; Langmore et al., 2002; Mazuc et al., 2003; Onbaşılar and Aksoy, 2005; Raouf et al., 2006; Smith et al., 2005). In Japanese quail, frequent changes in the group composition of breeding females are thought to reflect increased social densities and lead to elevated plasma corticosterone concentrations (Guibert et al., 2010). In contrast, Japanese quail females housed in pairs had higher circulating androgen levels and tended to have higher circulating corticosterone levels than group-housed females (Langen et al., 2017). Such effects of the social environment on female physiology and body mass and condition may affect their ability to invest in reproduction, resulting in changes in the quality or quantity of eggs produced or the quality or quantity of the offspring (Christians, 2002; Drent and Daan, 1980; Lim et al., 2014; Ronget et al., 2018; Sockman et al., 2006). Studies have reported both positive and negative correlations between measures of reproduction and circulating androgens (positive: Cain and Ketterson, 2012; Langmore et al., 2002; Sandell, 2007; negative: de Jong et al., 2016; López-Rull and Gil, 2009; Rutkowska et al., 2005; Rutkowska and Cichoń, 2006; Veiga and Polo, 2008) and glucocorticoids (positive: Bonier et al., 2009b; Burtka et al., 2016; Ouyang et al., 2011, 2013; negative: Angelier et al., 2010; Bonier et al., 2009b; Ouyang et al., 2011, 2013; Silverin, 1986; Vitousek et al., 2014).

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The influence of the social environment on female physiology and reproductive investment can, in turn, lead to effects on offspring development and fitness. Kaiser et al. (2003) found in guinea pigs (*Cavia aperea*), for instance, that maternal social instability resulted in decreased maternal plasma androgen concentrations, and affected offspring behaviour and physiology. Daughters of socially unstable mothers were masculinized in their behaviour and had increased plasma androgen concentrations during adulthood, whereas sons were infantilized. In American red squirrels (*Tamiasciurus hudsonicus*), higher maternal social densities increased maternal corticosterone and offspring growth rates (Dantzer et al., 2013). In Japanese quail (*C. japonica*), maternal social instability reduced offspring growth during the first weeks of life (Guibert et al., 2010). Maternal effects on growth and physiology may influence offsprings' future reproduction, as an individual's reproductive performance often depends on its body condition and/or endocrine status (Burtka et al., 2016; Correa et al., 2011; de Jong et al., 2016; Devries et al., 2008; Festa-Bianchet et al., 1998; López-Rull and Gil, 2009; Milenkaya et al., 2015; Ouyang et al., 2011, 2013; Rutkowska et al., 2005; Veiga and Polo, 2008). However, the adaptive significance of maternal effects induced by social stimuli is still insufficiently understood.

In the present study, we investigated the potential interactive effects of the maternal and offspring social environment in Japanese quail. Females of the parental (P0) generation were housed in pairs (one female and one male) or in groups (three females and one male) and allowed to reproduce (Langen et al., 2017). The females of the offspring (F1) generation were similarly housed in either pairs of two females or groups of four females, with daughters from the two maternal conditions evenly allocated to the two F1 social conditions. This allowed us to investigate the effects of the P0 social environment, the F1 female's own social environment, and the interaction of these environments on physiology (body mass and circulating levels of corticosterone and androgens) and reproduction (egg production, egg mass, fertilization rates, hatching success and offspring mass). We assessed the sensitivity of the F1 female's hypothalamic–pituitary–adrenal (HPA) axis using a standardized restraint stress challenge (Wingfield et al., 1995) and assessed the responsiveness of the hypothalamic–pituitary–gonadal (HPG) axis using a gonadotropin-releasing hormone (GnRH) challenge (Jawor et al., 2006; Peluc et al., 2012). This enabled us to investigate whether effects on reproductive performance reflect physiological changes during reproduction (e.g. Angelier et al., 2010; Bonier et al., 2009b; Burtka et al., 2016; Cunningham et al., 1987; Ouyang et al., 2011, 2013).

Adaptive effects of the maternal social environment should prepare their offspring for the social environment anticipated by the mother's social experience. We therefore expected F1 female offspring to become heavier and reproduce better under social conditions matching the maternal environment compared with the female offspring housed under mismatched social conditions. Social density and group size are frequently positively correlated with circulating androgen or corticosterone levels (Cunningham et al., 1987; Mazuc et al., 2003; Onbaşılar and Aksoy, 2005; Raouf et al., 2006; Smith et al., 2005). This would suggest higher plasma androgen or corticosterone concentrations in group-housed females compared with pair-housed females. However, as we previously found that female Japanese quail housed in pairs had higher circulating androgen levels and tended to have higher circulating corticosterone levels compared with females housed in groups (Langen et al., 2017), we expected that the reverse might also be found.

MATERIALS AND METHODS

Ethics statement

All experimental procedures were approved by the North Rhine-Westphalia State Agency for Nature, Environment and Consumer Protection (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen), Recklinghausen, Germany (licence number 84-02.04.2013-A127). Animal facilities were approved for keeping and breeding Japanese quail for research purposes by the local government authority responsible for health, veterinary and food monitoring (Gesundheits-, Veterinär- und Lebensmittelüberwachungsamt Bielefeld, Germany).

Origin of the parental generation

The eggs from which the parental generation hatched were provided by the INRA in Nouzilly, France [Experimental unit 1295 (UE PEAT) and UMR 85, Physiologie de la Reproduction et des Comportements, INRA-CNRS-IFCE-Université de Tours, Val de Loire Center, Nouzilly, France]. The eggs were laid by females from a non-selected control line, bred next to quail lines selected for low or high social reinstatement (Mills and Faure, 1991).

Social environments

Females were housed under two different social conditions shortly before sexual maturity. P0 females were housed in pairs (one female with one male) or in groups (three females with one male), whereas F1 females were housed in pairs (two females, one offspring from each of the P0 treatments) or in groups (four females, two offspring from each of the P0 treatments). All experiments and testing described for P0 generation females (Langen et al. 2017), and for F1 generation females from hatching to the beginning of the experimental social conditions (Langen et al. 2018) have been published previously. The birds were placed in the experimental social conditions at the age of 29 days in the P0 generation (see Langen et al., 2017) and 24 days in the F1 generation (Fig. 1), about 2 weeks before the onset of egg laying. At these initial time points the birds were unfamiliar with each other. Siblings and half-siblings (in the P0) or cousins (in the F1) were never housed in the same cage. F1 males (n=15, all offspring from the P0 pair treatment) were housed in single cages and only encountered females for mating. Males were not housed with females in the F1 generation to avoid injury to the females that could result from high copulation frequency when housed in pairs (see Langen et al., 2017).

In the P0 generation, 17 pair-housed females and 20 group-housed females produced F1 offspring (see Langen et al., 2017). Thirteen of the pair-housed females and 13 of the group-housed females produced the 53 daughters used in the current F1 experiment. These F1 females were allocated to 16 pairs and seven groups, mixing offspring from the two maternal treatments where possible, so that females from both were exposed to the same current social treatment (see also Table S1). We thus created four different treatments in the F1 generation, representing all combinations of the P0 and F1 social conditions: daughters from pair-housed mothers housed in pairs ($P_{P0}P_{F1}$, n=16), daughters from pair-housed mothers housed in groups ($P_{P0}G_{F1}$, n=11), daughters from group-housed mothers housed in groups ($G_{P0}G_{F1}$, n=13), and daughters from group-housed mothers housed in pairs ($G_{P0}P_{F1}$, n=13). Three pair cages and three group cages contained females that were not used for the experimental tests, but served as cage mates for the experimental birds (see also Table S1). These seven females were the offspring of P0 birds that had been excluded from the experiments due to aggression (for more information, see Langen et al., 2017). For details on sample sizes, see Table 1.

Journal of Experimental Biology (2019) 222, jeb187005. doi:10.1242/jeb.187005

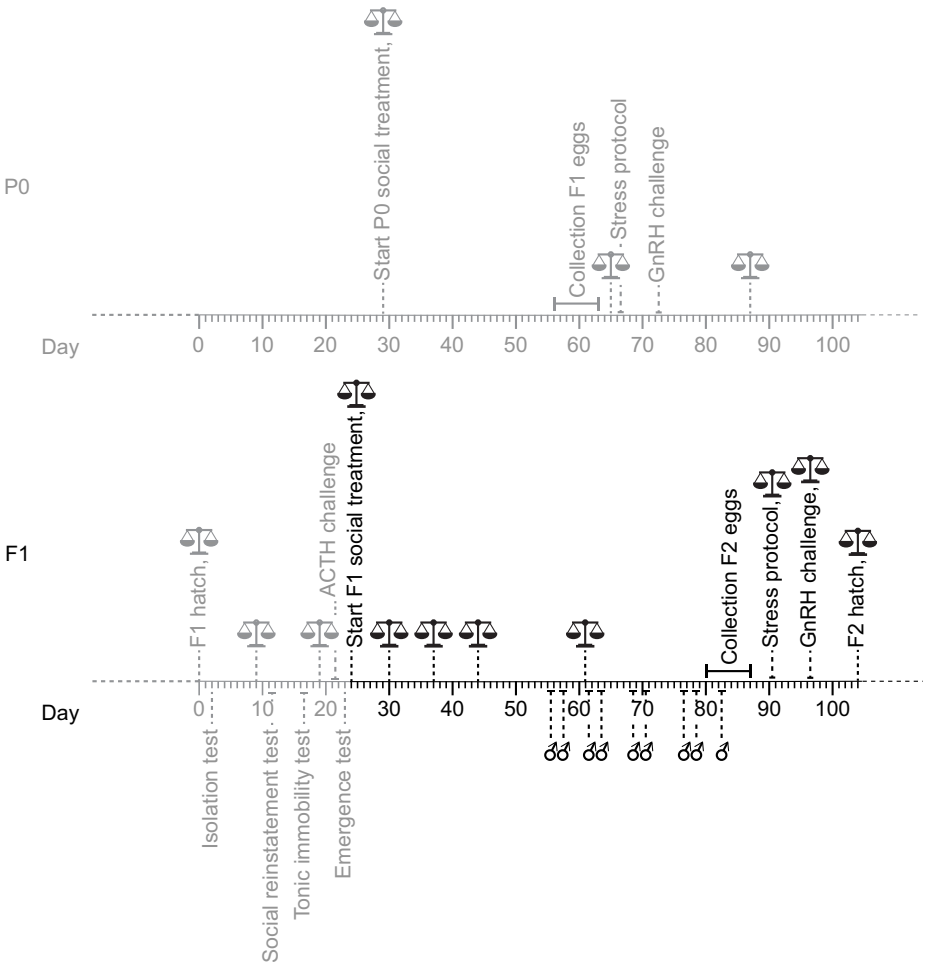

Fig. 1. Timeline of P0 and F1 generation experimental procedures. Measurements in grey are not presented here, but some of these are published elsewhere (for more information, see Langen et al., 2017, 2018). Scale symbols indicate when animals were weighed. ♂ indicates when females and males were brought together for mating.

Because of aggression, we had to separate 11 pairs and four groups in the F1 generation over the course of the experiment. Of the 11 pairs, 10 were separated using a wire mesh so that visual, acoustic and limited tactile interaction were still possible, and they were kept in our experiment. One pair was completely separated and removed from the experiment because one of the females had wounds that were unlikely to heal within a few days, constituting a pre-established humane endpoint. The four groups had to be fully separated because it was not possible to use a wire mesh in their cage to keep them apart and allow visual, acoustic and tactile interaction. We included only data from before the separation of the one pair and the four groups, and after separation all females from the respective cages were excluded. In addition, for some females, measurements were not included in certain analyses owing to missing samples (for one female, cortisol measurements from the stress protocol were missing; for two females, androgen measurements from the GnRH challenge were missing because blood sampling failed; for one female, no reproductive measures could be calculated because she did not lay any eggs). Therefore, each measurement had a different sample size (for exact sample sizes, see Tables 1, 2 and Figs 2, 4 and 5). For more details on when the birds were separated, see Table S1.

Table 1. Experimental groups and sample sizes

Maternal social environment	P0 females	Own social environment	F1 females (P0 mothers)
P_{P0}	13	P^a_{F1}	16 (11[c])
		G^b_{F1}	11 (9[c])
G_{P0}	13	P^a_{F1}	13 (10[d])
		G^b_{F1}	13 (9[d])

Number of females in the two P0 social treatments and in the four combinations of F1 social treatments.
[a]Housed in 16 F1 pair cages; [b]housed in 7 F1 group cages; [c]7 P0 pair-housed mothers contributed to both F1 pairs and F1 groups; [d]6 P0 group-housed mothers contributed to both F1 pairs and F1 groups.

Animal husbandry

All birds were housed in two adjacent rooms in the P0 generation (see Langen et al., 2017) and three adjacent rooms in the F1 generation (two rooms for the females and one room for the males). All rooms had artificial lighting and ambient temperature, with a minimum temperature of 20°C. Main lights were set to a 14 h:10 h light:dark cycle (lights on at 05:00 h), except for the first day and night after hatching when lights remained on for 24 h. Cages never faced each other to prevent visual contact between birds from different cages, but acoustic and olfactory communication was possible.

In the P0 generation, pairs were kept in cages measuring 75×80×40 cm, and groups were kept in cages measuring 150×80×40 cm. The adult F1 females were all kept in cages measuring 150×80×40 cm, irrespective of their social conditions. Males were housed in cages measuring 75×80×40 cm. The cage floors were covered with wood shavings, and all cages contained a sand bath and one shelter hut per bird. Food (GoldDott Hennenmehl, Derby Spezialfutter, Münster, Germany) and water were provided *ad libitum*. On a weekly basis, the standard diet was supplemented with mealworms and shell grit.

Table 2. Sample sizes for F1 egg laying rates, egg mass, fertilization, hatching success and F2 offspring body mass at hatching

Maternal and own social environment	F1 females contributing to egg data	Eggs laid	Eggs fertilized	Eggs hatched	F1 females with F2 offspring hatching	F2 offspring
$P_{P0}P_{F1}$	15	93	73	24	13	24
$G_{P0}P_{F1}$	12	79[a]	48	21	8	20[b]
$G_{P0}G_{F1}$	6	38	23	15	5	15
$P_{P0}G_{F1}$	6	36	23	11	4	11

[a]Because of to an oversight only 77 eggs were weighed. [b]21 chicks hatched, but one chick was excluded from the mass measurements because of birth defects.

Females were weighed before they were housed in their adult social condition on day 24, and on days 30, 37, 44, 61, 90 and 97.

Mating

Females of the F1 generation were housed in single-sex groups but had temporary access to males for mating (see Fig. 1). In each mating session, males and females were together for 20 min. Fifteen males, all sons of pair-housed females, were used in total, and females were always paired with the same unrelated male (not sharing the same grandparents). Each male was paired with four different females, one from each combination of the P0 and F1 social conditions, except for one male that was only paired to $P_{P0}P_{F1}$ females. On days 55–56, males were introduced into the home cages of the females and allowed to mate for 20 min. As the males were unable to copulate with all of the two or four females in a cage within such a short time period, we paired males with one female at a time in subsequent mating sessions. Each female was paired twice a week, and each male was paired with the same two females within a day but in alternating order. Furthermore, we began the mating sessions with a different male and female every day so that the pairing order was randomized for males as well as females. On days 57–58, 61–62 and 63–64, females and males were paired for 5 min at a time in a neutral mating cage between 08:00 h and 17:00 h. Thereafter, on days 68–69, 70–71, 76–77, 78–79 and 82–83, females were introduced to their male's home cage and left together with their male for 20 min between 10:00 h and 12:30 h.

Egg collection for the F2 generation, incubation and hatching

Eggs for the F2 generation were collected on days 80–87. All eggs were stored at 16°C until the end of the collection period (storage time ranging from 1 to 7 days) when incubation started. All eggs were incubated at the same time in a HEKA-Euro-Lux II incubator (HEKA-Brutgeräte, Rietberg, Germany). Incubation was done in complete darkness to avoid the effects of light on development (Archer and Mench, 2014). From incubation day 1 to day 14, the temperature was set at 37.8°C, humidity at 55%, and the eggs were turned every 2 h. Eggs were candled after 9 days of incubation to identify embryonic development. Non-fertilized eggs were removed (see Table 2 for number of eggs and fertilization). From day 15 onwards, the incubation temperature was set at 37.5°C, the humidity at 75%, and the eggs were no longer turned. After 15 days of incubation, eggs were placed in separate compartments (5.5×5.5×5 cm) on hatching trays. The individual compartments allowed us to identify which chick hatched from which egg. The compartment walls were made of transparent Plexiglas and the bottom of each hatching tray was made of mesh wire, allowing air flow and olfactory and acoustic communication between the chicks.

All eggs hatched after 17±1 days (mean±range) of incubation. Hatchlings were removed from the incubator once their feathers had dried (ca. 2 h after hatching) and weighed to the nearest 0.1 g. A blood sample (maximum 50 µl or ∼0.5% of body mass) was taken for assignment of parentage. Samples <0.8% of body mass do not appear to have long-term effects on adult or developing birds (Sheldon et al., 2008). Blood sampling was done by piercing the jugular vein with a sterile 27-gauge needle and collecting the blood in heparinized capillaries (BRAND, Wertheim, Germany).

Parentage assignment

F2 hatchling blood was centrifuged for 10 min at 2000 ***g***. Blood cells were diluted 1:2 with phosphate buffered saline (10 mmol l^{-1} PBS+6 mmol l^{-1} EDTA, pH 7.4) and stored at −20°C. We used a small sample of blood from the stress protocol or GnRH challenge from the adult F1 females. Genomic DNA was obtained by a phenol/chloroform or Chelex extraction (Walsh et al., 1991). Parentage was manually assigned after genotyping all parents and offspring at 22 microsatellite loci using fluorescently labelled primers, as described previously (Langen et al., 2017).

Stress protocol and GnRH challenge

The stress protocol and the GnRH challenge were performed after collecting the F2 generation eggs to exclude effects on reproduction. The stress protocol took place on days 90–91. All birds were tested between 09:20 h and 12:30 h, and corticosterone levels did not change significantly during that period (χ^2_1=0.30, P=0.58). After catching the birds and removing them from their home cages, a blood sample was taken within 3 min to determine baseline plasma corticosterone concentrations by puncturing the ulnar vein with a sterile needle and collecting 200–300 µl blood in heparinized capillaries (BRAND). After baseline samples were taken, the birds were restrained for 10 min by placing them in a cotton bag (Ecotone, 25×30 cm). A second blood sample was taken after the 10-min restraint period to determine the female's corticosterone response. In total, 2×200–300 µl blood was collected on the days of the stress protocol and the GnRH challenge, or ∼0.18–0.28% of body mass at those ages.

The GnRH challenge took place on days 96–97 while all females were laying eggs and thus assumed to be responsive to GnRH (Jawor et al., 2006; Peluc et al., 2012). All birds were tested between 09:25 h and 12:30 h. As in the stress protocol, birds were caught, and a blood sample was taken from the ulnar vein within 3 min to determine baseline plasma androgen concentrations. After the baseline sample was taken, the females were injected in the pectoral muscle with 5 µg (based on Peluc et al., 2012) chicken GnRH-I (H-3106, APC number 54-8-23, CAS No: 47922-48-5, Bachem, Bubendorf, Switzerland; formerly also sold as Sigma-L0637) dissolved in 50 µl PBS, and returned to their home cages. Thirty minutes post-injection, the birds were caught again and a second blood sample was taken to determine the female's plasma androgen concentration in response to GnRH.

Hormone analysis

Blood samples from the stress protocol and the GnRH challenge were kept on ice for a maximum of 2 h after sampling and then

Journal of Experimental Biology (2019) 222, jeb187005. doi:10.1242/jeb.187005

centrifuged for 10 min at 2000 ***g***. Following centrifugation, plasma was collected and frozen at −20°C.

Plasma corticosterone concentrations were determined using a commercial corticosterone radioimmunoassay kit (07-102102, MP Biomedicals, Orangeburg, SC, USA). Cross-reactivity of the kit antibody was 0.34% for desoxycorticosterone, 0.1% for testosterone, and less than 0.1% for all other steroids tested (as reported by the manufacturer). Samples were measured alongside quail plasma samples from other experiments and were distributed over 10 assays with an average intra-assay coefficient of variation (CV) of 4.78%, and an inter-assay CV of 7.13% (based on a chicken plasma pool and two kit controls measured in duplicate in each assay). Across assays, samples were balanced for treatment.

Plasma androgen concentrations were determined using a commercial testosterone enzyme immunoassay kit (DES6622, Demeditec Diagnostics, Kiel, Germany). Cross-reactivity of the kit antibody was 23.3% for 5α-dihydrotestosterone, 1.6% for androstenedione and less than 0.1% for other tested steroids (as reported by the manufacturer). Samples were measured alongside quail plasma samples from other experiments and were distributed over nine assays with an average intra-assay CV of 4.38% (based on all plasma samples measured in duplicate), and an inter-assay CV of 13.82% (based on two control plasma pools measured in each of the nine assays). Across assays, samples were balanced for treatment.

Statistical analysis

Data were analysed using R 3.4.3 (https://www.r-project.org), package lme4 (Bates et al., 2015). General linear mixed models were fitted for body mass at all measurement points, body mass around egg collection, egg mass, F2 body mass at hatching and plasma hormone levels. Analysis of egg laying rate (eggs per female per day between day 80 and day 87), fertilization and hatching success was done using generalised linear mixed models with a binomial error distribution and logit link function. To control for the non-independence of F1 offspring from the same P0 mother, we always included P0 mother as a random effect. We also included a random effect of F1 female nested within P0 mother for repeated measurements from the same F1 female (body mass, egg laying rate, fertilization and hatching success, and plasma hormone levels).

All models included P0 social environment, F1 social environment and their interaction as fixed effects. Models analysing plasma hormones included an additional fixed effect of sample, and its two-way and three-way interaction with the P0 and F1 social environment. For the GnRH challenge, all females received the same amount of GnRH, without adjustment of the dosage for individual body mass. To investigate whether body mass affected circulating androgen levels or the response to the GnRH injection, we ran additional GnRH models including female mass as a covariate. Models analysing body mass included a linear, quadratic and cubic effect of age in days ($day+day^2+day^3$) to model the non-linear relationship between age and mass. In addition, the two-way and three-way interactions between ($day+day^2+day^3$) and the P0 and F1 social environment were included. The female's age in days was centred on the mean age within our dataset by subtracting 45 from each age. The intercept and main effects of the models therefore represent the estimated body mass at day 45.

We tested whether effects on F1 female mass could explain differences in F2 egg mass by including F1 female body mass at day 90 (close to the period of egg collection) as a covariate in the model. Similarly, we included egg mass as a covariate in models testing effects on F2 body mass at hatching. We also tested whether effects on body mass at hatching depended upon offspring sex.

We started out with the full models, including all interactions, and then stepwise excluded all non-significant predictors or interactions (P>0.05), except for the main parameters of interest, i.e. social treatment, age in days ($day+day^2+day^3$; for body mass) and sample number (for hormonal responses: baseline and post-restraint or post-GnRH injection samples). Interactions were always excluded before the main effects involved in the interaction. We determined the significance of fixed effects using likelihood ratio tests, comparing the models with and without the parameter of interest. Distributions of model residuals were visually assessed for normality and homoscedasticity using histograms and Q–Q plots. Plasma corticosterone concentrations were $\log_{10}$ transformed to achieve normality. The results of all models are reported in Tables S2–S5, and the dataset used for analyses is reported in Table S1.

RESULTS

Body mass, egg mass and offspring mass

Females housed in groups increased body mass faster than females housed in pairs [own social environment×($day+day^2+day^3$): χ^2_3=21.94, P<0.001; Fig. 2A]. In addition, there was a significant effect of the interaction between the P0 maternal social environment and F1 own social environment on female body mass (χ^2_1=4.14, P=0.04). The P0 social environment on its own or in interaction with age did not affect female body mass (χ^2<0.46, P>0.58). The dataset was split according to maternal social environment and by day of weighing for further *post hoc* testing. This analysis revealed that F1 group housing had a positive effect on body mass increase in daughters of pair-housed mothers and no effect on body mass increase in daughters of group-housed mothers (see Table S2 for more details). Furthermore, splitting the dataset by day revealed that the interaction effect between the maternal and own social environment on female mass was significant at days 37 and 44, with a non-significant trend at day 61. There was no significant interaction effect on days 24, 30, 90 and 97. From day 44 onwards, the F1 females' own social environment significantly affected their body mass at each time point, with group-housed females being heavier than pair-housed females. Detailed results of the *post hoc* tests can be found in Table S2. Towards the end of the experiment, the separations of certain cages (see Materials and Methods) might have biased our results because of the exclusion of heavier or lighter females. We therefore repeated the body mass analysis, including only data up to day 61 when most females were still included. In this analysis, the effect of the interaction between the maternal and own social environment was borderline non-significant (χ^2_1=3.78, P=0.052). The effect of own social environment on body mass increase was not significant [own social environment×($day+day^2+day^3$): χ^2_3=6.05, P=0.11; see Table S2 for more details].

At day 90, close to the period of egg collection for the F2 generation, females housed in groups were significantly heavier than females housed in pairs (χ^2_1=6.44, P=0.011; Fig. 2B) and there was no longer an effect of the interaction with the P0 treatment (χ^2_1=0.34, P=0.56). Additionally, females housed in groups laid heavier eggs than females housed in pairs (χ^2_1=6.02, P=0.014; Fig. 2C) and the F2 offspring of females housed in groups were heavier at hatching than offspring of females housed in pairs (χ^2_1=12.53, P<0.001; Fig. 2D). The P0 social environment did not affect egg mass or F2 body mass at hatching, and did not interact with the effects of the F1 social environment (all χ^2_1<1.36, all P>0.24; all χ^2_3<4.51, all P>0.21; Fig. 2; Tables S2–S3). We also found no sex differences in F2 offspring body mass at hatching, and no effect of the interaction between F2 sex with the P0 maternal and the F1 own social environment (Table S3).

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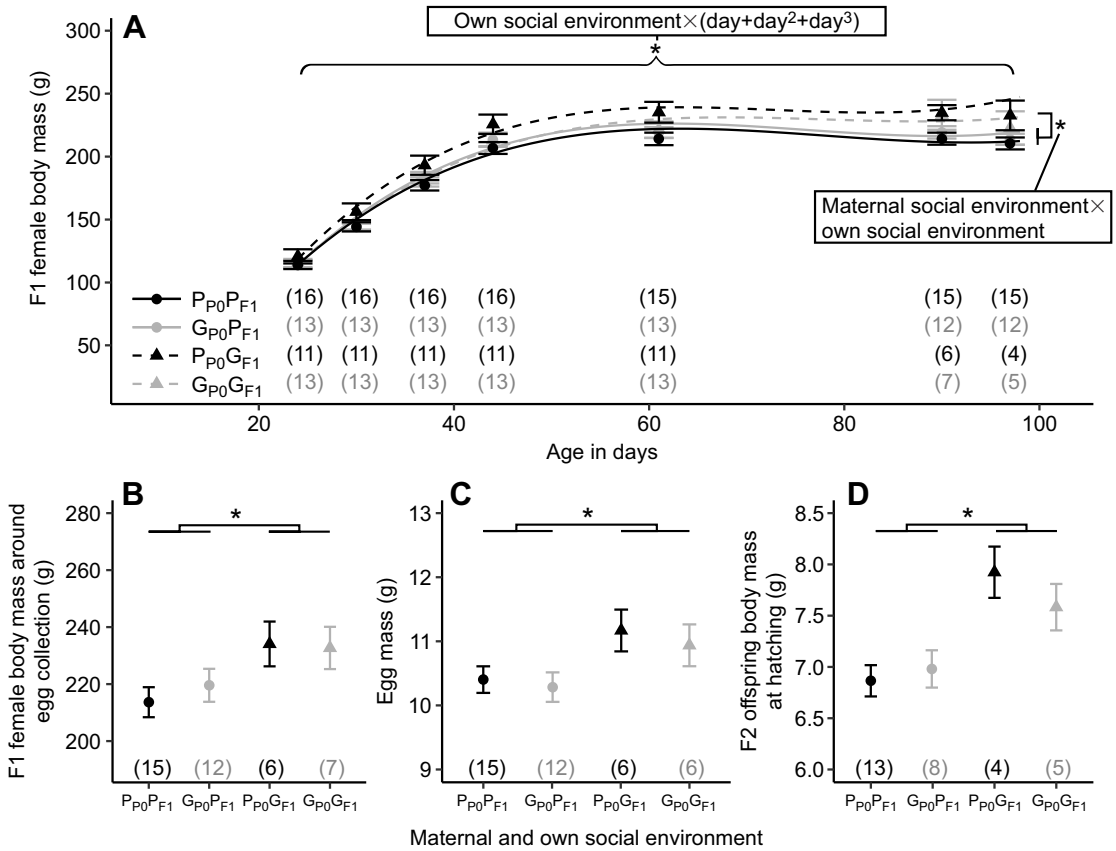

Fig. 2. Female body mass, body mass around egg collection, egg mass and F2 offspring body mass at hatching. (A) Female body mass. F1 females housed in groups (triangles and dashed lines) increased body mass faster than F1 females housed in pairs (circles and solid lines). In addition, F1 group housing had a positive effect on body mass, but only in daughters of pair-housed females, not of group-housed females. (B) Mean female body mass around egg collection (day 90). Females housed in groups were significantly heavier than females housed in pairs. There was no effect of the maternal social environment or its interaction with the female's own social environment. (C) Egg mass. Females housed in groups laid significantly heavier eggs than females housed in pairs. There was no effect of the maternal social environment or its interaction with the female's own social environment. (D) F2 offspring body mass. Females housed in groups had significantly heavier F2 offspring than females housed in pairs. There was no effect of the maternal social environment or its interaction with the female's own social environment. Data shown in A are raw means±1 s.e.m., with lines indicating model predictions. Data shown in B–D are estimated means±1 s.e.m. Numbers in parentheses indicate the number of F1 females included (for numbers of F2 offspring, see Table 2). *P<0.05 (see Materials and Methods for details on which statistical methods were used). $P_{P0}P_{F1}$, daughters from pair-housed mothers housed in pairs; $P_{P0}G_{F1}$, daughters from pair-housed mothers housed in groups; $G_{P0}G_{F1}$, daughters from group-housed mothers housed in groups; $G_{P0}P_{F1}$, daughters from group-housed mothers housed in pairs.

Egg mass was significantly positively correlated with F1 female body mass at day 90 (χ^2_1=5.59, P=0.02; Fig. 3A; Table S3). When controlling for female body mass at day 90, the effect of the female's own social environment on egg mass was no longer significant (χ^2_1=2.45, P=0.12; Table S3), suggesting that the effect of the F1 social environment on egg mass was mediated by effects on female body mass. Similarly, F2 body mass at hatching was significantly positively correlated with egg mass (χ^2_1=135.61, P<0.001; Fig. 3B; Table S3), and when controlling for egg mass, the effect of the female's own social environment on F2 body mass at hatching was no longer significant (χ^2_1=1.39, P=0.24; Table S3). This suggests that the effect of the F1 social environment on F2 body mass at hatching was mediated by the effects on egg mass.

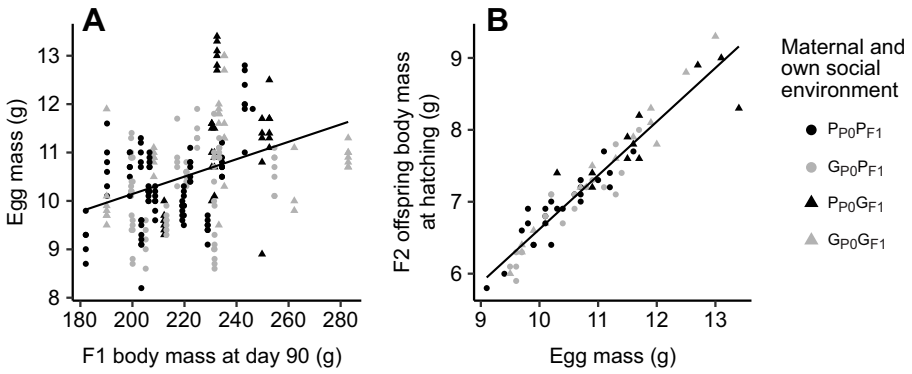

Fig. 3. Relationships between F1 female body mass around egg collection, egg mass and F2 offspring mass at hatching. (A) Relationship between F1 female mass around egg collection (day 90) and egg mass. There was a significant positive correlation between F1 female body mass and the mass of their eggs. (B) Relationship between egg mass and body mass of the F2 offspring at hatching. Egg mass was positively correlated with F2 offspring body mass at hatching.

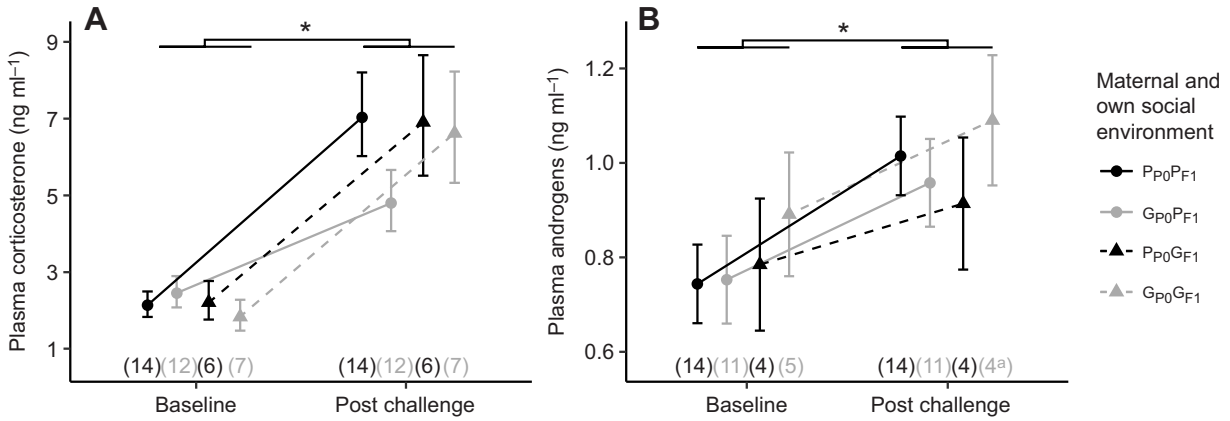

Fig. 4. Female plasma hormone levels. (A) Plasma corticosterone concentrations of F1 females at 90–91 days before and after being restrained for 10 min (back-transformed from $\log_{10}$). Significantly increased plasma corticosterone concentrations were observed following 10 min of restraint, but there was no effect of the maternal or own social environment or their interaction on the increase, or on average plasma corticosterone concentrations. (B) Plasma androgen concentrations of F1 females at 96–97 days before and after injection with 5 µg GnRH. Androgen concentrations increased significantly in response to the GnRH injection, but there was no effect of the maternal or own social environment or their interaction on the increase, or on average plasma androgen concentrations. Data shown are the estimated means±1 s.e.m. Numbers in parentheses indicate the number of F1 females included. [a]Insufficient plasma for one $G_{P0}G_{F1}$ female in the response sample. *P<0.05 (see Materials and Methods for details on which statistical methods were used).

Stress protocol and GnRH challenge

Females responded to the 10 min of restraint with a significant increase in plasma corticosterone concentrations (χ^2_1=53.24, P<0.001; Fig. 4A), but the corticosterone response did not differ between females from different maternal or own social environments (maternal social environment×sample: χ^2_1=1.69, P=0.19; own social environment×sample: χ^2_1=1.69, P=0.19; Fig. 4A). There was also no effect of the interaction between the maternal and own social environment on the female's stress response (maternal social environment×own social environment×sample: χ^2_1=2.33, P=0.13; Fig. 4A). Average plasma corticosterone concentrations were not affected by the female's own social environment, the maternal social environment, or their interaction (all χ^2_1<0.64, all P>0.43; Fig. 4A; Table S4).

GnRH injections resulted in a significant increase in plasma androgen concentrations (χ^2_1=26.43, P<0.001; Fig. 4B), but the androgen response to the GnRH challenge did not differ between females from different maternal or own social environments (maternal social environment×sample: χ^2_1=0.22, P=0.64; own social environment×sample: χ^2_1=0.96, P=0.33; Fig. 4B). The female's androgen response to GnRH was not affected by the interaction between the maternal and own social environment (maternal social environment×own social environment×sample: χ^2_1=0.72, P=0.40; Fig. 4B). Average plasma androgen concentrations were not affected by the female's own social environment, the maternal social environment, or their interaction (all χ^2_1<0.55, all P>0.46; Fig. 4B; Table S4). Female body mass at the time of the GnRH challenge significantly affected their response to the GnRH injection (sample×F1 body mass: χ^2_1=7.80, P=0.005; Table S4). *Post hoc* tests on the dataset split by sample revealed that there was a non-significant trend for female body mass to positively affect baseline androgen levels (F1 body mass: χ^2_1=3.30, P=0.07; Table S4), but there was no effect of female body mass on response androgen levels (F1 body mass: χ^2_1=0.89, P=0.35; Table S4). Including female body mass in the GnRH models did not change the effects of the maternal or own social environment. We therefore excluded female body mass from the final models to avoid potential confounding effects caused by multicollinearity (as female body

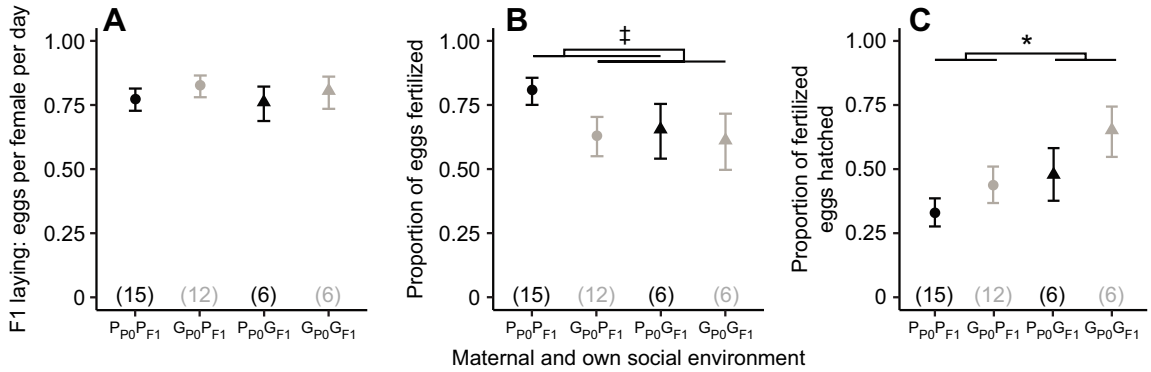

Fig. 5. Female reproduction. (A) Number of eggs laid per female per day. Egg laying rates were not affected by the maternal or own social environment or their interaction. (B) Proportion of eggs fertilized. There was a small non-significant effect of the maternal social environment, with offspring from pair-housed mothers laying slightly more fertilized eggs than offspring from group-housed mothers. Fertilization success was not affected by own social environment or the interaction between the maternal and own social environment. (C) Hatching success of fertilized eggs. Hatching success was higher for females housed in groups than for females housed in pairs. Hatching success was not affected by the maternal social environment or its interaction with the female's own social environment. Data shown are the estimated means±1 s.e.m. (back-transformed from logit). Numbers in parentheses indicate the number of F1 females included (for number of eggs, see Table 2). *P<0.05; ‡0.05<P<0.1 (see Materials and Methods for details on which statistical methods were used).

mass was affected by the social environment, another predictor in the model).

Reproduction

Egg laying rates (eggs per female per day) were not affected by the maternal social environment (χ^2_1=0.89, *P*=0.35; Fig. 5A), the F1 female's own social environment (χ^2_1=0.11, *P*=0.75; Fig. 5A), or the interaction between the maternal and own social environment (χ^2_1=0.01, *P*=0.92; Fig. 5A). Offspring from pair-housed mothers laid slightly more fertilized eggs than offspring from group-housed mothers, but the difference did not reach statistical significance (χ^2_1=2.89, *P*=0.09; Fig. 5B). There was no effect of the F1 female's own social environment (χ^2_1=1.08, *P*=0.30; Fig. 5B) or of the interaction between the maternal and own social environment on fertilization success (χ^2_1=0.77, *P*=0.38; Fig. 5B).

The hatching success of fertilized eggs was higher for females housed in groups than for females housed in pairs (χ^2_1=4.07, *P*=0.04; Fig. 5C). The maternal social environment and its interaction with the female's own social environment did not affect hatching success of fertilized eggs (χ^2_1=2.63, *P*=0.11 and χ^2_1=0.13, *P*=0.72, respectively; Fig. 5C). Overall hatching rates (the proportion of all eggs collected for the F2 generation that hatched, i.e. including non-fertilized eggs) were not affected by the female's own social environment, the maternal social environment, or their interaction (all χ^2_1<1.88, all *P*>0.17; Table S5).

DISCUSSION

This study is the first, to our knowledge, to test for evidence of adaptive maternal effects and the underlying mechanisms in relation to social group size in a match–mismatch experiment across two generations in Japanese quail. Body mass of the F1 females was affected by their own social environment, as females housed in groups increased body mass faster and ended up heavier compared with pair-housed females. Notably, however, body mass of the F1 females also depended on the interaction between the maternal and own social environment, which resulted from an additional positive effect on mass in daughters of P0 pair-housed females only when they were housed in F1 groups. This interaction effect on body mass disappeared by the time eggs for the F2 were collected (day 90). This suggests that group-housed offspring of pair-housed females increased body mass at an earlier age than offspring of group-housed females that caught up later. There was no effect of the P0 social environment on F1 body mass before the F1 social treatment started (see also Langen et al., 2018). The positive effect on offspring body mass in the mismatched environment, at least for offspring of pair-housed females, contradicts the expectation of an adaptive maternal effect, as it does not suggest that offspring perform better in the environment matching the maternal one. A non-adaptive explanation may be a silver spoon effect resulting from increased maternal investment of pair-housed mothers resulting in a stronger positive effect of the group environment on their mass compared with the effect on offspring of group-housed mothers (Marshall and Uller, 2007; Uller et al., 2013). We have previously found no evidence of a difference in P0 maternal investment as egg mass and yolk androgen levels did not differ between groups (Langen et al., 2017). However, P0 females housed in pairs had higher circulating androgen levels (Langen et al., 2017), which may be associated with differences in other aspects of egg quality. To explain why a maternal effect may be context dependent, it has been suggested that more competitive or otherwise challenging conditions may be required to detect maternal effects on offspring phenotype (Benowitz-Fredericks et al., 2015; Verboven et al., 2003). Offspring of pair-housed females may thus respond more strongly than offspring of group-housed females to the stimulating effect of the social group environment. While, overall, our results thus do not suggest an adaptive effect, they emphasize the importance of investigating maternal effects under different environmental conditions in the offspring.

The interaction effect of the P0 and the F1 social environment on female mass disappeared by the time eggs for the F2 were collected, and at that point only the positive effect of the current group size on female body mass remained. This effect can explain the larger egg size and hatching success, and a positive maternal effect on F2 hatchling body mass for group-housed females. The positive effects of group housing on egg mass and offspring body mass at hatching can ultimately have important fitness consequences because both are important predictors of offspring growth and survival (Krist, 2011; Williams, 1994). Our results thus strongly suggest that there is additional scope for adaptive maternal effects in relation to group size in Japanese quail and that the observed effects of the social environment on body mass have important consequences for egg and offspring quality.

The effects of pair housing versus group housing on females and their offspring differed between the P0 and F1 generations. In the P0 generation, we previously found that female plasma androgen and corticosterone concentrations were affected, but there were no effects on body mass, reproduction or F1 offspring mass at hatching (Langen et al., 2017). In contrast, the social environment of the F1 females affected body mass, reproduction and F2 offspring body mass, but not circulating androgen and corticosterone concentrations or the hormonal response to challenges. A possible explanation for these differences is that the sex ratios within pairs and groups differed between the generations. Whereas males were continuously present in the female's social environment in the P0 generation, they were housed separately from the females in the F1 generation, and male–female interaction was only possible during the mating sessions. Pair housing in the P0 generation probably resulted in more social stimulation by the male, leading to elevated female plasma androgen levels and a trend of higher plasma corticosterone (Langen et al., 2017). This effect of the male presence might have been diluted in the P0 group-housed environment. In the F1 generation, female exposure to the male was standardized, explaining the absence of a treatment difference in endocrine parameters and a stronger effect of group size on female mass. The contrasting effects of the P0 and the F1 social treatments may have been caused not only by the differences in sex ratio, but also by slight differences in timing between the P0 and F1 generation in the onset of the social treatments (day 29 in the P0 generation versus day 24 in the F1 generation), the age at which females were first mated, the timing of sampling (for details see Fig. 1) and the number of females present.

F1 females that were housed in groups increased more in body mass than pair-housed females and were heavier around the time of egg collection. This was unexpected, as a negative correlation between group size or social density and growth or body mass has been reported in many animal species, including Japanese quail, probably owing to increased competition for resources (Asghar Saki et al., 2012; Keeling et al., 2003; Onbaşılar and Aksoy, 2005). However, increased social stimulation can also lead to increased body mass, as demonstrated in European starlings (*Sturnus vulgaris*) (Witter and Goldsmith, 1997), potentially because higher levels of social stimulation can increase food intake rates (Beauchamp, 1998; Hoppitt and Laland, 2008; Tolman, 1964). As we did not measure female body composition, we do not know

whether differences in body mass between the social treatments were the result of an overall increase in body mass or the result of increased mass of specific tissues, such as the reproductive organs, which might be an explanation for the larger F2 egg mass, offspring body mass and hatching success. Increased body mass is generally expected to be beneficial under higher social densities because it may increase female competitive abilities (Clutton-Brock and Huchard, 2013; Stockley and Bro-Jørgensen, 2011), and our results indicate that it can lead to increased reproductive investment, in line with previous findings (Christians, 2002; Drent and Daan, 1980; Lim et al., 2014; Ronget et al., 2018; Sockman et al., 2006). Other proximate explanations may be changes in feed conversion or metabolic rate, potentially in combination with maternal effects. Both (maternal) corticosterone and testosterone levels may affect metabolism and body mass (Dantzer et al., 2013; Groothuis et al., 2005; Sapolsky et al., 2000). However, even though the P0 maternal circulating hormone levels were affected, we did not find differences in yolk hormone deposition in previous studies (Langen et al., 2017) or plasma steroids of the F1 females in the current study. Moreover, F1 group- and pair-housed females did not show different hormonal responses to the challenges, meaning that our measurements do not suggest that differences in body mass were linked to hormonal differences. As we were unable to determine social status, it is also unclear whether differences in social hierarchy within pairs and groups may have contributed to the effects on body mass and other parameters. Finally, some cages had to be removed from the experiment owing to aggression of some females, and we cannot exclude the possibility that this may have contributed to the effect on body mass, because the growth trajectories of the removed females may have differed. When analysing only body mass data until day 61, when most cages were still included, the model estimated a similar effect of F1 group housing on body mass to that observed in the full dataset, even though it was less clear and no longer significant.

Egg laying rates were not affected by the maternal or own social environment and fertilization success was not affected by own social environment, but daughters from pair-housed mothers had a non-significantly higher proportion of fertilized eggs than daughters from group-housed mothers. This effect was small and did not reach statistical significance, but a similar trend to higher fertility of pair-housed mothers was previously seen in the P0 generation (Langen et al., 2017). This suggests a genetic or non-genetic maternal effect on fertility that should be further investigated as it is a core fitness component.

Effects on female mass and reproduction in the F1 generation did not correspond with changes in female endocrine parameters, suggesting that effects of the social environment on female mass and reproduction were not mediated by differences in female plasma androgens and corticosterone in our experiments. Conversely, in the P0 generation, hormone differences did not lead to reproductive differences. Other studies report non-significant, positive, and negative correlations between circulating androgens or glucocorticoids and measures of reproduction, e.g. egg production (Gerlach and Ketterson, 2013; Veiga and Polo, 2008), hatching success (de Jong et al., 2016; Schmidt et al., 2009) and number of fledglings (Burtka et al., 2016; O'Neal et al., 2008; Ouyang et al., 2011), suggesting that the relationships are non-linear and can change across contexts and over time (Bonier et al., 2009a; Hau and Goymann, 2015; Ouyang et al., 2011, 2013). Moreover, it is important to note that owing to the exclusion of some groups as a result of aggression, the sample size of group-housed females for the endocrine measurements became rather small at the end of the study when hormone measurements were taken (ranging from four to seven females).

Conclusions

We have shown that offspring development is affected by the maternal social environment, the offspring's own social environment and the interaction of the two. The effects differ according to the trait of interest and time point of measurement. While F1 group housing generally had a positive effect on body mass, there was an additional positive effect on F1 body mass seen only when offspring of pair-housed females were housed in groups, suggesting that differences in P0 maternal investment modulated offspring response to its own environment. This result emphasizes the importance of considering the context under which maternal effects are studied and lends some support to the idea that maternal effects may be revealed better under more challenging or stimulating conditions. The interaction effect between the maternal and offspring social environment disappeared over time, to be replaced by the effects of the F1 generation's own social environment, which resulted in a maternal effect on the F2 generation that was independent of the P0 social environment. The observed changes in body mass in the F1 and F2 generations are likely to have important consequences for performance and fitness, but their adaptive significance remains unclear. Effects of social group size on female physiology and reproduction differed between the P0 and the F1 generation, most likely because the adult sex ratio did not remain constant over the generations. This might have led to differences in social stimulation between pairs and groups of both generations, potentially explaining why the effects of the matched and mismatched social conditions did not confirm expectations. Future studies into the adaptive maternal effects of the social environment and their underlying proximate mechanisms should assess the fitness consequences for offspring in more depth. Furthermore, the importance of the type of social stimuli experienced (e.g. group size, adult sex ratio, intrasexual and intersexual interactions) should be investigated in more detail.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: E.M.A.L., V.C.G.-J., N.v.E.; Methodology: E.M.A.L., V.C.G.-J., N.v.E.; Validation: E.M.A.L., V.C.G.-J., N.v.E.; Formal analysis: E.M.A.L., V.C.G.-J., N.v.E.; Investigation: E.M.A.L., V.C.G.-J., N.v.E.; Resources: V.C.G.-J., N.v.E.; Data curation: E.M.A.L.; Writing - original draft: E.M.A.L.; Writing - review & editing: E.M.A.L., V.C.G.-J., N.v.E.; Visualization: E.M.A.L.; Supervision: V.C.G.-J., N.v.E.; Project administration: V.C.G.-J.; Funding acquisition: V.C.G.-J.

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Supplementary information

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Table S1

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Table S2. General linear mixed model of effects of the maternal and F1 own social environment on female body mass.

Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**205.200**	**4.620**	**35.870**	**44.403**			
Day * maternal social environment * own social environment[a]	0.002	0.254	286.900	0.006			
Day^2 * maternal social environment * own social environment[a]	0.022	0.013	286.900	1.667	4.51	3	0.21
Day^3 * maternal social environment * own social environment[a]	$-4.62*10^{-4}$	$3.13*10^{-4}$	287.100	-1.474			
Day * maternal social environment[a]	0.030	0.126	286.900	0.236			
Day^2 * maternal social environment[a]	0.003	0.007	286.900	0.459	0.46	3	0.93
Day^3 * maternal social environment[a]	$-5.64*10^{-5}$	$-1.54*10^{-4}$	286.900	-0.366			
Day * own social environment	**0.168**	**0.128**	**287.000**	**1.313**			
Day^2 * own social environment	**-0.002**	**0.007**	**286.900**	**-0.225**	**21.94**	**3**	**< 0.001**
Day^3 * own social environment	**$7.25*10^{-5}$**	**$1.57*10^{-4}$**	**287.100**	**0.460**			
Maternal social environment * own social environment	**-13.260**	**6.391**	**34.090**	**-2.076**	**4.14**	**1**	**0.04**
Day	**2.215**	**0.082**	**286.900**	**26.884**			**< 0.001**
Day^2	**-0.086**	**0.004**	**286.700**	**-19.739**			**< 0.001**
Day^3	**$-8.97*10^{-4}$**	**$9.93*10^{-5}$**	**286.800**	**9.038**			**< 0.001**
Maternal social environment	**3.648**	**6.506**	**34.100**	**0.561**			**0.58**
Own social environment	**14.370**	**4.723**	**42.220**	**3.043**			**0.004**
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID[b]	**85.45**	**9.244**					
Maternal ID	**159.47**	**12.628**					
Residual	**120.24**	**10.966**					
Post-hoc tests; split by maternal social environment:							
Offspring of pair-housed mothers							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**205.800**	**5.636**	**15.520**	**36.513**			
Day * own social environment	**0.165**	**0.169**	**146.600**	**0.975**			
Day^2 * own social environment	**-0.013**	**0.009**	**146.400**	**-1.466**	**20.77**	**3**	**< 0.001**
Day^3 * own social environment	**$3.08*10^{-4}$**	**$2.08*10^{-4}$**	**146.700**	**1.482**			

Day	2.194	0.103	146.600	21.265			<0.001
Day^2	-0.083	0.005	146.300	-15.353			<0.001
Day^3	$8.42*10^{-4}$	$1.24*10^{-4}$	146.400	6.814			<0.001
Own social environment	15.530	3.685	31.050	4.214			<0.001
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID[b]	28.370	5.327					
Maternal ID	342.450	18.506					
Residual	102.540	10.126					
Offspring of group-housed mothers							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	208.800	4.209	32.390	49.601			
Day * own social environment	0.164	0.191	140.400	0.858			
Day^2 * own social environment	0.009	0.010	140.400	0.925	6.55	3	0.09
Day^3 * own social environment	$1.48*10^{-4}$	$2.35*10^{-4}$	140.400	-0.632			
Day	2.241	0.129	140.300	17.373			<0.001
Day^2	-0.090	0.007	140.300	-13.013			<0.001
Day^3	$9.66*10^{-4}$	$1.57*10^{-4}$	140.300	6.172			<0.001
Own social environment	-1.544	5.494	34.330	-0.281			0.78
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID[b]	122.610	11.073					
Maternal ID	37.150	6.095					
Residual	134.390	11.593					
Post-hoc tests; split by own social environment							
Pair-housed F1 females							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	202.400	4.154	19.270	48.727			
Day	2.215	0.077	168.700	28.641			
Day^2	-0.086	0.004	168.700	-21.038	476.62	3	< 0.001
Day^3	$8.98*10^{-4}$	$9.31*10^{-5}$	168.700	9.640			
Maternal social environment	5.322	5.883	17.940	0.905	0.81	1	0.37
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID[b]	85.190	9.230					
Maternal ID	101.430	10.070					
Residual	105.850	10.290					
Group-housed F1 females							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	218.000	6.102	19.490	35.731			

Day	**2.383**	**0.106**	**117.600**	**22.496**			
Day^2	**-0.088**	**0.006**	**117.600**	**-15.855**	**331.68**	**3**	**< 0.001**
Day^3	**9.68*10^{-4}**	**1.32*10^{-4}**	**117.700**	**7.315**			
Maternal social environment	**-11.430**	**8.379**	**17.280**	**-1.364**	**1.77**	**1**	**0.18**
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID[b]	**63.110**	**7.944**					
Maternal ID	**244.420**	**15.634**					
Residual	**141.150**	**11.881**					
Post-hoc tests; split by day							
Day 24							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**116.295**	**3.561**	**28.387**	**32.662**			
Maternal social environment	**-2.113**	**4.779**	**23.580**	**-0.442**	**0.2**	**1**	**0.66**
Own social environment	**3.441**	**2.893**	**34.772**	**1.189**	**1.39**	**1**	**0.24**
Maternal social environment * own social environment[a]	-2.135	5.811	34.585	-0.367	0.13	1	0.72
Random effects							
	Variance	Std.Dev.					
Maternal ID	**95.840**	**9.790**					
Residual	**88.530**	**9.409**					
Day 30							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**149.121**	**4.292**	**27.343**	**34.741**			
Maternal social environment	**-5.379**	**5.861**	**23.980**	**-0.918**	**0.83**	**1**	**0.36**
Own social environment	**4.349**	**2.894**	**31.810**	**1.503**	**2.19**	**1**	**0.14**
Maternal social environment * own social environment[a]	-8.599	5.729	31.905	-1.501	2.19	1	0.14
Random effects							
	Variance	Std.Dev.					
Maternal ID	**171.760**	**13.106**					
Residual	**83.810**	**9.155**					
Day 37							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**180.357**	**4.879**	**33.334**	**36.968**			
Maternal social environment	**3.955**	**7.085**	**35.612**	**0.558**			**0.58**
Own social environment	**13.919**	**5.018**	**33.335**	**2.774**			**0.009**
Maternal social environment * own social environment	**-16.558**	**7.219**	**34.714**	**-2.294**	**5.00**	**1**	**0.03**
Random effects							

	Variance	Std.Dev.					
Maternal ID	**177.000**	**13.300**					
Residual	**135.300**	**11.630**					
Day 44							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**208.473**	**5.542**	**32.531**	**37.617**			
Maternal social environment	**6.778**	**8.053**	**34.895**	**0.842**			**0.41**
Own social environment	**19.030**	**5.775**	**32.545**	**3.295**			**0.002**
Maternal social environment * own social environment	**-16.671**	**8.306**	**33.983**	**-2.007**	**3.88**	**1**	**0.049**
Random effects							
	Variance	Std.Dev.					
Maternal ID	**223.700**	**14.960**					
Residual	**179.900**	**13.410**					
Day 61							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**220.301**	**5.640**	**28.335**	**39.064**			
Maternal social environment	**-2.699**	**7.355**	**21.428**	**-0.367**	**0.13**	**1**	**0.71**
Own social environment	**10.442**	**5.143**	**36.334**	**2.030**	**3.97**	**1**	**0.046**
Maternal social environment * own social environment[a]	-18.76	9.835	36.251	-1.907	3.49	1	0.06
Random effects							
	Variance	Std.Dev.					
Maternal ID	**187.900**	**13.710**					
Residual	**280.700**	**16.750**					
Day 90							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**214.755**	**4.930**	**24.713**	**43.559**			
Maternal social environment	**3.520**	**6.680**	**19.953**	**0.527**	**0.28**	**1**	**0.60**
Own social environment	**16.726**	**6.318**	**32.297**	**2.648**	**6.44**	**1**	**0.011**
Maternal social environment * own social environment[a]	-7.336	12.550	32.250	-0.585	0.34	1	0.56
Random effects							
	Variance	Std.Dev.					
Maternal ID	**56.580**	**7.522**					
Residual	**321.820**	**17.939**					
Day 97							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**210.825**	**4.915**	**21.503**	**42.898**			
Maternal social	**1.260**	**6.873**	**18.242**	**0.183**	**0.03**	**1**	**0.86**

environment							
Own social environment	**14.973**	**6.970**	**29.798**	**2.148**	**4.31**	**1**	**0.04**
Maternal social environment * own social environment[a]	-12.447	13.816	29.726	-0.901	0.8	1	0.37
Random effects							
	Variance	Std.Dev.					
Maternal ID	**63.770**	**7.985**					
Residual	**290.630**	**17.048**					
Including only data until day 61 (when most cages were still together):							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**215.200**	**4.612**	**35.970**	**46.656**			
Day * maternal social environment * own social environment[a]	0.137	0.635	211.100	0.215			
Day^2 * maternal social environment * own social environment[a]	0.015	0.025	211.100	0.628	5.42	3	0.14
Day^3 * maternal social environment * own social environment[a]	-0.001	0.002	211.100	0.633			
Day * maternal social environment[a]	0.235	0.320	211.100	0.735			
Day^2 * maternal social environment[a]	-0.010	0.012	211.100	-0.833	0.90	3	0.83
Day^3 * maternal social environment[a]	-9.47*10-4	0.001	211.100	-0.871			
Day * own social environment	0.349	0.321	211.100	1.086			
Day^2 * own social environment	-0.008	-0.012	211.100	-0.603	6.05	3	0.11
Day^3 * own social environment	-5.50*10^{-4}	0.001	211.100	-0.505			
Maternal social environment * own social environment	**-11.920**	**6.019**	**33.010**	**-1.980**	**3.78**	**1**	**0.052**
Day	**3.374**	**0.162**	**211.100**	**20.812**			**<0.001**
Day^2	**-0.139**	**0.006**	**211.100**	**-22.127**			**<0.001**
Day^3	**-0.003**	**5.50*10^{-4}**	**211.100**	**-6.064**			**<0.001**
Maternal social environment	**2.455**	**6.464**	**33.780**	**0.380**			**0.71**
Own social environment	**12.700**	**4.177**	**31.960**	**3.041**			**0.005**
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID[b]	**75.400**	**8.683**					
Maternal ID	**168.620**	**12.985**					
Residual	**78.430**	**8.856**					
Post-hoc tests; split by maternal social environment:							
Offspring of pair-housed mothers							
Fixed effects							

	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**215.100**	**5.642**	**16.790**	**38.127**			
Day	**3.245**	**0.233**	**107.100**	**13.931**			
Day^2	**-0.134**	**0.009**	**107.200**	**-14.773**	**361.07**	**3**	**<0.001**
Day^3	**-0.003**	**7.90*10^{-4}**	**107.100**	**-3.602**			
Own social environment	**11.990**	**3.213**	**15.000**	**3.733**	**10.31**	**1**	**0.001**
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID^b	**33.720**	**5.807**					
Maternal ID	**326.380**	**18.066**					
Residual	**82.450**	**9.080**					
Offspring of group-housed mothers							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**218.200**	**4.080**	**30.540**	**53.488**			
Day	**3.509**	**0.224**	**104.000**	**15.653**			
Day^2	**-0.145**	**0.009**	**104.000**	**-16.674**	**356.94**	**3**	**<0.001**
Day^3	**-0.004**	**7.61*10^{-4}**	**104.000**	**-5.501**			
Own social environment	**-0.166**	**4.606**	**21.720**	**-0.036**	**0.001**	**1**	**0.97**
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID^b	**105.100**	**10.252**					
Maternal ID	**49.710**	**7.050**					
Residual	**73.600**	**8.579**					
Post-hoc tests; split by own social environment							
Pair-housed F1 females							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**211.100**	**4.114**	**20.640**	**51.296**			
Day	**3.215**	**0.197**	**115.000**	**16.290**			
Day^2	**-0.136**	**0.008**	**115.000**	**-17.732**	**398.72**	**3**	**<0.001**
Day^3	**-0.003**	**0.001**	**115.000**	**-4.617**			
Maternal social environment	**4.514**	**5.687**	**17.750**	**0.794**	**0.62**	**1**	**0.43**
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID^b	**116.67**	**10.801**					
Maternal ID	**68.99**	**8.306**					
Residual	**63.58**	**7.973**					
Group-housed F1 females							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**227.100**	**6.075**	**21.570**	**37.387**			
Day	**3.564**	**0.260**	**96.000**	**13.707**			
Day^2	**-0.143**	**0.010**	**96.000**	**-14.231**	**312.08**	**3**	**<0.001**
Day^3	**-0.004**	**0.001**	**96.000**	**-4.124**			

Maternal social environment	**-11.110**	**8.127**	**17.220**	**-1.367**	**1.77**	**1**	**0.18**
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID[b]	**67.49**	**8.215**					
Maternal ID	**227.08**	**15.069**					
Residual	**91.41**	**9.561**					

Estimates are given on the original scale. Maternal and F1 pair-housing is coded as 0, group-housing is coded as 1. Factors included in the final model are presented in bold.

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

[b] F1 ID nested within maternal ID.

Table S3. General linear mixed model of effects of the maternal and F1 own social environment on egg mass and offspring mass at hatching.

Effects on egg mass:					
Fixed effects					
	Estimate	SE	χ^2	df	p
(Intercept)	**10.412**	**0.193**			
Maternal social environment * own social environment[a]	-0.115	0.556	0.04	1	0.84
Maternal social environment	**-0.153**	**0.257**	**0.35**	**1**	**0.55**
Own social environment	**0.711**	**0.278**	**6.02**	**1**	**0.01**
Random effects					
	Variance	Std.Dev.			
Maternal ID : F1 ID[b]	**0.595**	**0.772**			
Maternal ID	**0.000**	**0.000**			
Residual	**0.258**	**0.508**			
Including F1 female mass at day 90 as a covariate:					
Fixed effects					
	Estimate	SE	χ^2	df	p
(Intercept)	**6.902**	**1.341**			
Maternal social environment * own social environment[a]	0.003	0.502	0.00	1	0.995
Maternal social environment	**-0.158**	**0.264**	**0.33**	**1**	**0.56**
Own social environment	**0.439**	**0.275**	**2.45**	**1**	**0.12**
F1 female mass at day 90	**0.016**	**0.006**	**5.59**	**1**	**0.02**
Random effects					
	Variance	Std.Dev.			
Maternal ID : F1 ID[b]	**0.430**	**0.655**			
Maternal ID	**0.091**	**0.302**			
Residual	**0.258**	**0.508**			
Effects on offspring mass at hatching:					
Fixed effects					
	Estimate	SE	χ^2	df	p
(Intercept)	**6.926**	**0.145**			
Maternal social environment * own social	-0.261	0.543	0.23	1	0.63

environment* F2 sex					
Maternal social environment * F2 sex	-0.138	0.262	0.27	1	0.60
Own social environment* F2 sex	-0.115	0.269	0.18	1	0.67
Maternal social environment * own social environment[a]	-0.457	0.413	1.19	1	0.28
Maternal social environment	**-0.032**	**0.199**	**0.03**	**1**	**0.87**
Own social environment	**0.830**	**0.211**	**12.53**	**1**	**< 0.001**
F2 sex	-0.116	0.131	0.78	1	0.38
Random effects					
	Variance	Std.Dev.			
Maternal ID : F1 ID[b]	**0.179**	**0.423**			
Maternal ID	**$2.40*10^{-16}$**	**$1.55*10^{-8}$**			
Residual	**0.228**	**0.477**			

Including egg mass as a covariate:					
Fixed effects					
	Estimate	SE	χ^2	df	p
(Intercept)	**-0.675**	**0.353**			
Maternal social environment * own social environment[a]	0.137	0.117	1.36	1	0.24
Maternal social environment	**-0.036**	**0.078**	**0.21**	**1**	**0.65**
Own social environment	**0.077**	**0.065**	**1.39**	**1**	**0.24**
Egg mass	**0.730**	**0.033**	**135.61**	**1**	**< 0.001**
Random effects					
	Variance	Std.Dev.			
Maternal ID : F1 ID[b]	**0.000[c]**	**0.000[c]**			
Maternal ID	**0.013**	**0.112**			
Residual	**0.042**	**0.205**			

Estimates are given on the original scale. Maternal and F1 pair-housing is coded as 0, group-housing is coded as 1. Factors included in the final model are presented in bold.

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

[b] F1 ID nested within maternal ID.

[c] variance parameters estimated as zero in the model.

Table S4. General linear mixed model of effects of the maternal and F1 own social environment on female plasma hormone levels in the restraint stress protocol and the GnRH challenge.

Female plasma corticosterone levels in the restraint stress procotol:					
Fixed effects					
	Estimate	SE	χ^2	df	p
(Intercept)	**0.364**	**0.055**			
Time of day[a]	0.420	0.762	0.30	1	0.58
Maternal social environment * own social environment * sample[a]	0.288	0.186	2.32	1	0.13
Maternal social environment * own social environment[a]	0.008	0.118	0.005	1	0.95
Own social environment * sample[a]	0.126	0.096	1.69	1	0.19
Maternal social environment * sample[a]	-0.121	0.092	1.69	1	0.19
Maternal social environment	**-0.056**	**0.067**	**0.64**	**1**	**0.42**
Own social environment	**0.012**	**0.059**	**0.04**	**1**	**0.85**
Sample	**0.451**	**0.047**	**53.24**	**1**	**< 0.001**
Random effects					
	Variance	Std.Dev.			
Maternal ID : F1 ID[b]	**0.005**	**0.072**			
Maternal ID	**0.008**	**0.091**			
Residual	**0.043**	**0.208**			
Female plasma androgen levels in the GnRH challenge:					
Fixed effects					
	Estimate	SE	χ^2	df	p
(Intercept)	**0.751**	**0.076**			
Maternal social environment * own social environment * sample[a]	0.136	0.159	0.72	1	0.40
Maternal social environment * sample[a]	-0.032	0.069	0.22	1	0.64
Maternal social environment * own social environment[a]	0.161	0.194	0.55	1	0.46
Own social environment * sample[a]	-0.079	0.080	0.96	1	0.33
Maternal social environment	**0.020**	**0.104**	**0.04**	**1**	**0.85**
Own social environment	**0.044**	**0.100**	**0.17**	**1**	**0.68**
Sample	**0.223**	**0.035**	**26.43**	**1**	**< 0.001**
Random effects					
	Variance	Std.Dev.			
Maternal ID : F1 ID[b]	**0.045**	**0.213**			
Maternal ID	**0.018**	**0.135**			
Residual	**0.020**	**0.143**			
Including female mass as a covariate:					
Fixed effects					
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**-0.744**	**0.559**	**41.512**	**-1.331**			
Maternal social environment * own social environment * sample * F1 body mass[a]	-0.002	0.007	33.208	-0.271	0.07	1	0.79
Maternal social environment * own social environment * F1	-0.007	0.010	33.460	-0.743	0.54	1	0.46

body mass[a]							
Maternal social environment * sample * F1 body mass[a]	-0.003	0.003	33.205	-0.869	0.75	1	0.39
Maternal social environment * own social environment * sample[a]	0.087	0.145	33.714	0.602	0.36	1	0.55
Own social environment * sample * F1 body mass[a]	0.002	0.003	33.206	0.692	0.48	1	0.49
Own social environment * sample[a]	-0.012	0.076	33.677	-0.152	0.02	1	0.88
Maternal social environment * F1 body mass[a]	-0.002	0.005	33.058	-0.497	0.20	1	0.66
Maternal social environment * own social environment[a]	0.074	0.120	34.477	0.370	0.14	1	0.71
Maternal social environment * sample[a]	-0.038	0.062	33.429	-0.612	0.37	1	0.54
Own social environment * F1 body mass[a]	-0.007	0.005	33.987	-1.491	2.15	1	0.14
Sample * F1 body mass	**-0.005**	**0.002**	**33.230**	**-2.964**	**7.80**	**1**	**0.005**
Maternal social environment	**0.030**	**0.089**	**34.150**	**0.332**	**0.11**	**1**	**0.74**
Own social environment	**-0.046**	**0.106**	**34.463**	**-0.492**	**0.17**	**1**	**0.68**
F1 body mass	**0.007**	**0.003**	**41.387**	**2.685**			**0.006**
Sample	**1.273**	**0.356**	**33.220**	**3.579**			**0.001**
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID[b]	**0.058**	**0.241**					
Maternal ID	**$1.17*10^{-15}$**	**$3.42*10^{-8}$**					
Residual	**0.016**	**0.127**					
Post-hoc tests; split by sample:							
Baseline							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**-0.560**	**0.621**	**33.440**	**-0.901**			
Maternal social environment	**0.037**	**0.113**	**17.108**	**0.326**	**0.10**	**1**	**0.75**
Own social environment	**-0.005**	**0.122**	**29.814**	**-0.045**	**0.002**	**1**	**0.97**
F1 body mass	**0.006**	**0.003**	**33.770**	**2.104**	**3.30**	**1**	**0.07**
Random effects							
	Variance	Std.Dev.					
Maternal ID	**0.015**	**0.123**					
Residual	**0.078**	**0.280**					
Post challenge							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	**0.531**	**0.488**	**33.000**	**1.089**			
Maternal social environment	**0.011**	**0.083**	**33.000**	**0.134**	**0.02**	**1**	**0.89**
Own social environment	**-0.051**	**0.101**	**33.000**	**-0.504**	**0.25**	**1**	**0.62**
F1 body mass	**0.002**	**0.002**	**33.000**	**0.950**	**0.89**	**1**	**0.35**
Random effects							
	Variance	Std.Dev.					

Maternal ID	**0.000[c]**	**0.000[c]**
Residual	**0.065**	**0.237**

Estimates for plasma corticosterone levels are given on the log10 scale, estimates for plasma androgen levels are given on the original scale. Maternal and F1 pair-housing is coded as 0, group-housing is coded as 1. The baseline sample (before restraint or the GnRH injection) is coded as 1, the post-restraint/challenge sample is coded as 2. Factors included in the final models are presented in bold.

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

[b] F1 ID nested within maternal ID.

[c] variance parameters estimated as zero in the model.

Table S5. Generalized linear mixed model of effects of the maternal and F1 own social environment on egg laying rates, fertilization success, hatching success of fertilized eggs and overall hatching rates (the proportion of all eggs collected for the F2 generation that hatched, i.e. including non-fertilized eggs).

Effects on egg laying rates:					
Fixed effects:					
	Estimate	SE	χ^2	df	p
(Intercept)	**1.241**	**0.244**			
Maternal social environment * own social environment[a]	-0.071	0.626	0.01	1	0.92
Maternal social environment	**0.309**	**0.327**	**0.89**	**1**	**0.34**
Own social environment	**-0.105**	**0.327**	**0.10**	**1**	**0.75**
Random effects:					
	Variance	Std.Dev.			
Maternal ID : F1 ID[b]	**0.008**	**0.090**			
Maternal ID	**0.111**	**0.334**			
Effects on fertilization success:					
Fixed effects:					
	Estimate	SE	χ^2	df	p
(Intercept)	**1.318**	**0.313**			
Maternal social environment * own social environment[a]	0.721	0.814	0.77	1	0.38
Maternal social environment	**-0.673**	**0.389**	**2.89**	**1**	**0.09**
Own social environment	**-0.432**	**0.416**	**1.08**	**1**	**0.30**
Random effects:					
	Variance	Std.Dev.			
Maternal ID : F1 ID[b]	**0.597**	**0.773**			
Maternal ID	**0.000[c]**	**0.000[c]**			
Effects on the hatching success of fertilized eggs:					
Fixed effects:					
	Estimate	SE	χ^2	df	p
(Intercept)	**-0.745**	**0.236**			
Maternal social environment * own social environment[a]	0.253	0.716	0.13	1	0.72
Maternal social environment	**0.536**	**0.324**	**2.63**	**1**	**0.11**
Own social environment	**0.744**	**0.356**	**4.07**	**1**	**0.04**
Random effects:					
	Variance	Std.Dev.			
Maternal ID : F1 ID[b]	**0.001**	**0.038**			
Maternal ID	**0.000[c]**	**0.000[c]**			
Effects on overall hatching rates:					
Fixed effects:					
	Estimate	SE	χ^2	df	p
(Intercept)	**-1.193**	**0.275**			
Maternal social environment * own social environment[a]	0.308	0.633	0.24	1	0.63
Maternal social environment	**0.188**	**0.334**	**0.32**	**1**	**0.57**
Own social environment	**0.446**	**0.318**	**1.88**	**1**	**0.17**

Random effects:		
	Variance	Std.Dev.
Maternal ID : F1 ID[b]	**0.000[c]**	**0.000[c]**
Maternal ID	**0.122**	**0.350**

Estimates are given on the logit scale. Maternal and F1 pair-housing is coded as 0, group-housing is coded as 1. Factors included in the final model are presented in bold.

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

[b] F1 ID nested within maternal ID.

[c] variance parameters estimated as zero in the model.