Coadaptation of offspring begging and parental provisioning: A role for prenatal maternal effects?

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1. Introduction

In species with parental care, the expression of offspring traits during early development is not only regulated by the offspring’s own genes in congruence with the external environment, but also indirectly by the genes expressed in its parents who shape the social environment of the offspring (Wolf et al., 1998; Kölliker, 2005; Räsänen and Kruuk, 2007). This social environment thus has a genetic basis, which facilitates the coevolution of traits via indirect genetic effects (Kölliker, 2005). The reciprocal interplay between parental food provisioning and offspring begging is one common example involving such indirect genetic effects. Each of these behaviors has a genetic basis regulating its own expression, but also affects the expression of the complementary behavior. These behaviors should therefore be genetically correlated (Wolf and Brodie, 1998; Kölliker et al., 2005). Such a correlation is expected to be stabilized by fitness costs to parents and offspring which arise when the levels of parental provisioning and offspring begging are mismatched. Consequently, offspring begging and parental provisioning are expected to be coadapted within families (Wolf and Brodie, 1998; Kölliker, 2005; Kölliker et al., 2005).

Coadaptation is typically investigated via cross-fostering to separate genetic factors and early environmental factors, providing evidence for covariation between parental provisioning and offspring begging, at least at the phenotypic level [(positive covariation: birds (Kölliker et al., 2000; Hinde et al., 2009, 2010; Estramil et al., 2013, 2015), mice (Hager and Johnstone, 2003), burying beetles (Lock et al., 2004, 2007); negative covariation: burrower bugs (Agrawal et al., 2001)]. Nevertheless, whether such covariation is truly genetic still remains largely unexplored (Hager and Johnstone, 2003; Curley et al., 2004; Estramil et al., 2014a). In addition, cross-fostering cannot control for prenatal maternal effects that result from factors which are deposited into the eggs or transferred via the placenta by the mother in order to adjust offspring phenotype to the environment they will experience post-hatching (Rossiter, 1996; Mousseau and Fox, 1998a, 1998b). In birds, prenatal maternal effects are extremely common, due to the strong dependency of embryonic development on the resources deposited into the sealed environment of the egg, and are often mediated by maternally-derived nutrients (Christians, 2002; Royle et al., 2002; Krist, 2011), dietary compounds (Isaksson et al., 2006; Szigeti et al., 2007) and/or hormones (e.g. Groothuis et al., 2005).
With regards to these resources, yolk androgens (e.g. testosterone) have been consistently found in the eggs of all avian species that have been analyzed (reviewed in Grootwuis et al., 2005). Most importantly, they are known to affect several offspring traits, including offspring begging and growth (Schwabl, 1996a; Grootwuis et al., 2005; Müller et al., 2010; Barnett et al., 2011; Noguera et al., 2013). Thus, prenatal factors—such as yolk androgens—can potentially be used by the mother to match the demands of her offspring to her own rate of provisioning therewith adjusting the development of her offspring to the prevailing environmental conditions (Grootwuis et al., 2005). Females may also have knowledge about the prospective levels of paternal care facilitating an adjustment of yolk hormone deposition to the paternal rate of provisioning. Such information may be conveyed via male (sexual) ornaments (e.g. Buchanan and Catchpole, 2000), representing a functional explanation for the findings that females deposit higher levels of yolk androgens when mated with attractive males (e.g. Gil et al., 2004; Kingma et al., 2009). Taken together, yolk androgens may be another causal mechanism facilitating co-adaptation by adjusting offspring demand to parental provisioning (Hinde et al., 2009, 2010; Estramil et al., 2014a), and so warrants further investigation.

The canary, our model species, is highly suitable to test this for the following reasons. First, yolk androgen levels have been shown to be affected by maternal condition and environmental and social conditions as experienced by the male before and during laying, such as food conditions and partner attractiveness (Schwabl, 1996b; Gil et al., 2004; Müller et al., 2012; Vergauwen et al., 2012). Secondly, there is evidence for a positive effect of yolk androgens on both offspring begging behavior and postnatal growth (Schwabl, 1996a; Grootwuis et al., 2005), in particular for testosterone, which is the predominant androgen in the yolk of canary eggs (Schwabl, 1993). Thirdly, covariation between parental provisioning and offspring begging has been also repeatedly reported (Hinde et al., 2009; Estramil et al., 2013, 2015).

We performed two cross-fostering experiments in order to test whether the deposition of testosterone into the yolk serves as a mechanism to match offspring begging to parental provisioning. In Experiment 1, we tested whether females deposit more yolk testosterone when the level of care provided by them and their partner is high. This should lead to a positive covariation between parental provisioning and offspring begging via the positive effects of testosterone on begging (Grootwuis et al., 2005). In Experiment 2, we investigated whether yolk testosterone deposition varies in response to experimentally-manipulated levels of offspring demand from the previous brood. This is based on the fundamental life history trade-off between current and future reproduction (Williams, 1966); that is, parental care is costly, meaning any investment in the current brood affects the potential to invest in a subsequent brood (Dijkstra et al., 1990; Schroeder et al., 2013). We expected females to deposit less testosterone into the egg yolk if the demand of their current offspring is high and vice versa. Furthermore, in this experiment, all females remained paired with the same male when laying a second clutch; thus, we also expected females to adjust yolk testosterone deposition to the levels of male care exhibited in order to match brood demand to paternal capacities.

2. Material and methods

2.1. Ethical note

Experiments complied with the current Flemish and Belgian institutional laws and were approved by the Ethical Committee for Animal Experiments of the University of Antwerp (license number 2011-07). Further, they were conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986 and the associated guidelines (EU Directive 2010/63/EU for animal experiments). Adequate measures were taken to minimize discomfort, and no deterioration in condition or abnormalities in appearance or behavior were observed, for instance as a consequence of handling.

2.2. Housing conditions

The experiments were performed in two consecutive years (Experiment 1: March 2012, Experiment 2: January 2011), but in identical settings. By the start of the experiments, birds were selected from our outbred canary stock population and housed in single-sex indoor aviaries under a fixed artificial photoperiod that mimicked the natural light: dark regime on a long day photoperiod (14:10 h light: dark), and a set room temperature range (19–24 °C) to stimulate reproductive activities. We provided quantities of canary seed mixture (Van Camp, Belgium), cuttlefish bone and fresh water ad libitum. This diet was supplemented with egg food (Van Camp, Belgium) twice a week. In both years, pairs were formed (Experiment 1 = 64 pairs, Experiment 2 = 42 pairs) after 4 weeks of acclimation, by assigning males and females to breeding cages at random. Before assigning each pair to a breeding cage, we measured female and male body mass to account for a potential effect of female condition and male quality. Each pair occupied a single breeding cage (50 × 64 × 40 cm, GEHU cages, The Netherlands) which was supplied with a nest box, nesting material, food (see above) and water. We additionally provided egg food and germinated seeds on a daily basis, once the nestlings had hatched. All birds were returned to large aviaries and kept under natural light conditions after each experiment was completed.

2.3. Experimental set-up

In Experiment 1, we aimed to test whether the amount of testosterone deposited in the egg yolk varies with the rate of parental provisioning of either parent or both parents. Thus, in order to measure testosterone deposition and parental provisioning of a respective pair, we needed to replace their clutch, which was collected for hormone analysis. For this reason, we created two groups. The first was an experimental group from which first clutches were collected (n = 27). Here, eggs were replaced by dummy eggs and frozen (at −20 °C) on the day they were laid. The second group donated their first clutches, 2 to 4 days after clutch completion, to the experimental group (n = 27). These donor clutches matched the clutches of the experimental group in both mass and size. Subsequently, once these donor eggs had hatched, we recorded the rate of parental food provisioning (Fig. 1).

In Experiment 2, we reciprocally cross-fostered complete first clutches between nests (i.e., dyads) and synchronized the within-brood hatching (for a description of the methodology see Estramil et al., 2013, 2014a). For all dyads, we collected data on offspring growth, offspring begging and parental provisioning. Subsequently, we collected the eggs of the second clutch, which was laid in direct succession, for analysis (n = 34 clutches). Again, eggs were replaced by dummy eggs and frozen (at −20 °C) on the day they were laid. We then tested whether females adjust yolk testosterone deposition for their second clutch in relation to: (a) the begging behavior and growth rate of nestlings of a previous (foster) brood, (b) their rate of provisioning as well as that of their partner when raising this previous brood, and (c) the difference between the begging behavior of their own (i.e., biological) nestlings raised by foster parents and the begging behavior of their foster nestlings (Fig. 1). This difference indicates whether parents were exposed to nestlings that were either more or less demanding than their own offspring, and thus whether their levels of care were enhanced or reduced accordingly (Estramil et al., 2013, 2015).

2.4. Experimental procedures

All methods were identical, unless indicated otherwise. At hatching (day = 0), nestlings were marked in different colors with non-toxic markers for individual recognition. We made daily measurements of nestling mass during the early phase of the nestling stage (i.e., day 0 to day 13). The nestling growth curve is sigmoidal (Ricklefs, 1968), but growth between day 0 and day 13 is rather linear. Consequently,
we calculated the slope of a linear regression (of age on nestling mass) for each nestling and then averaged the slopes per brood in order to estimate the average growth rate per nest (Hinde et al., 2009; Estramil et al., 2013, 2014b). The modal brood size at hatching was 4 (range: 2–4).

Due to cross-fostering, we measured offspring phenotype including begging behavior independently of the provisioning (or other phenotypic traits) of their biological parents, and parental provisioning independently of the phenotype of their biological offspring.

### 2.4.1. Parental provisioning

We videotaped the provisioning of food to 10-day-old foster nestlings as a measure of the postnatal social environment generated by the parents. To this end, we removed the food from each cage and fed all nestlings until satiation (Orluxe Handmix, Versele-Laga, Belgium) one hour before starting each video session in order to standardize the hunger levels among nestlings. Immediately before starting the recordings, we provided fresh egg food and germinated seeds to stimulate parents to start provisioning. The provisioning of food was videotaped for either 3 h (Experiment 1) or 2 h (Experiment 2) between 10:00 and 16:15 h, using Sony video cameras (DCR-SX 30). Room temperature varied between 22 and 24 °C. We analyzed the videos with The Observer XT 10 event logging software (Noldus, The Netherlands). In granivorous species, such as the canary, parents pre-digest the food in the crop before provisioning. Therefore, estimating the exact amount of food transferred off the crop was difficult, but the number of feeds per provisioning visit has been shown to be a good proxy for parental effort of captive granivorous bird species (Gilby et al., 2011). Consequently, we used the total number of dips of parental bills into the beaks of their nestlings (i.e., food transfers) as an estimate of parental provisioning (Müller et al., 2010). Provisioning can be either direct or indirect. Direct provisioning is when either parent feeds the nestlings directly. Indirect provisioning is when one parent (typically the female) is first fed by its partner — known as allofeeding — and then feeds its nestlings. Note that, during indirect provisioning, it is the partner who prepares and pre-digests the food. Food transfers provided by one of the parents after receiving food from its partner were recorded as (indirect) food transfers given by the partner, who did the food preparation and pre-digestion. Since females did not provide indirect dips during any of the video sessions, females provided an equal number of total and direct food transfers.

#### 2.4.2. Begging intensity

We performed begging tests according to previously established standard protocols (see Hinde et al., 2009; Estramil et al., 2013, 2014a). Briefly, at day 5, we removed the nest and food from each cage and fed all nestlings until satiation with Orluxe Handmix (Versele-Laga, Belgium). This allowed for standardization of hunger levels across all siblings. Two nestlings per nest were then tested by placing them singly into a central hole (2 cm in diameter, 1.5 cm deep) inside of a wooden test box (10 × 10 × 13 cm), which was filled with expandable polystyrene. Test boxes were then closed. We moved a maximum of 8 test boxes to a heated climate room (29–30 °C) and placed each box on a single table. After 90 min of food deprivation, we opened and immediately tapped each box 3 consecutive times with an iron rod. Thus, nestlings were exposed to both sound and light stimuli at the same time. Each begging test ended after 5 s of no begging. These trials were performed between 08:50 and 16:50 h, and were videotaped using Sony video cameras (DCR-SX 30). Immediately, after each round of trials, which usually took no more than 10 min to complete, we returned nestlings to their original cages and fed them until satiation with Orluxe Handmix. Video recordings were analyzed with Windows media player. To estimate nestling begging intensity, we scored begging posture every second (0 = not begging; 1 = gape open; 2 = gape open, head back; 3 = gape open, head back and neck stretched; 4 = same as 3, plus back vertical, and then summed all scores over the time period as in Kilner (2001).

#### 2.5. Hormone analysis

In order to obtain data on both mean clutch levels and changes across the laying order, we either collected full clutches (Experiment 1) or the first- and third-laid eggs of a clutch (Experiment 2). We
determined yolk testosterone concentrations following a protocol described previously (Goerlich et al., 2009). Briefly, yolks were weighed to the nearest 0.001 g. 1:1 diluted with demineralized water and homogenized thoroughly. We then weighed ca. 125 mg of each yolk mixture for extraction. Prior to the extraction we added ca. 5000 cpm radioactive-labeled testosterone to each sample in order to account for losses due to the extraction procedure. Each sample was extracted three times with 2.0 mL diethyl ether/petroleum benzine 70:30 (vol:vol). After vortexing (60 s), centrifuging (5 min, 2000 rpm, 4 °C) and snap freezing, the organic phase was decanted and the extract was dried under a stream of nitrogen. Then, 2.0 mL 70% methanol was added and the samples were vortexed until the complete dried pellet was dissolved. Samples were frozen at −20 °C overnight and then centrifuged (5 min, 2000 rpm, 4 °C), decanted, and dried. The pellet was redissolved in 400 μL phosphate-buffered saline with gelatine. Recoveries of the labeled testosterone were measured in a subsample of this solution, with recovery averaging 77%. Yolk testosterone concentration was determined using commercial Radioimmunoassay (RIA) kits (Spectria® T Coated-Tube RIA kit, Orion Diagnostica Espoo, Finland; detection limit: 0.16 ng/mL; antibody cross-reactivity: 100% testosterone, 2.6% α,β-dihydrotestosterone, and 1.7% androstenedione). Samples were distributed over two RIAs such that all samples from one experiment were measured within the same assay. The assay kits were validated by plotting serial dilutions of four random yolk samples against a standard curve. Standard curves went from 0.08 ng testosterone/mL to 20 ng testosterone/mL in both assays, and were measured in duplicate, as well as assay controls. RIA concentrations were corrected for initial yolk mass and calculated as pg/mg yolk. ‘Pools’ of yolk were used as external controls and intra-assay CV for testosterone was 2.4%.

As pointed out by Groothuis et al. (2005), it is as yet unknown whether hormone concentration or the total amount of hormone deposited is the most biologically relevant factor given that experimental studies manipulating total amount and concentration separately are still lacking (Groothuis et al., 2005) When considering female investment in terms of androgen deposition, the total amount should be considered. Consequently, we used the total amount of yolk testosterone (calculated by multiplying the testosterone concentration by the mass of the yolk) in the statistical analyses. The results for the total amount of testosterone were, however, virtually identical; thus, for simplicity, they are presented in the Supplementary material.

2.6. Statistical analysis

2.6.1. Experiment 1: Offspring matching via differential allocation of yolk testosterone

We used linear mixed models with either the yolk testosterone concentration or the total amount of yolk testosterone (see Supplementary material) as the response variable (n = 27 nests, 113 eggs). Clutch size, female body mass, male body mass, laying order (i.e., continuous variable), the number of food transfers provided towards a foster brood by both parents or else by either the female or the male and couple identity (i.e., random effect) were used as explanatory variables. We also applied a random slope model, but it reduced model fit and did not qualitatively alter the results, and therefore was not included.

The advantage of using the described mixed models is the possibility to include several factors. However, given the set-up of this experiment and the models used here, we do not expect causality with respect to parental provisioning. In fact, we are more interested in testing whether or not there is a correlation between the concentration or the amount of yolk testosterone and parental provisioning. Therefore, we additionally performed Pearson’s correlations between the number of food transfers and either the mean yolk testosterone concentration or the mean amount of yolk testosterone, for each case.

2.6.2. Experiment 2: Allocation of yolk testosterone in response to previously experienced offspring demand and parental investment

We used linear mixed models to test for carry-over effects of previous parental investment, which was experimentally manipulated by changing offspring demand, on yolk testosterone deposition (either concentration or total amount - see Supplementary material) as measured in a second, subsequent clutch (n = 34 nests, 67 eggs). The number of food transfers previously provided by both parents or else by the female or the male, clutch size (i.e., second clutch), egg position (i.e., 1st or 3rd egg), and couple identity (i.e., random effect) were included as explanatory variables. We repeated this analysis using the average begging intensity or average growth rate per brood as an explanatory variable instead of the number of food transfers (n = 34 nests, 67 eggs). Similar to what should be expected if parents would be raising more demanding offspring, those raising a higher number of offspring will likely incur higher costs if they respond to offspring demands, which may impinge on subsequent testosterone deposition. Therefore, we included brood size in the model in which the average begging intensity was used, since this was the only model in which there was no significant linearity between the explanatory variables. However, neither the concentration nor the total amount of testosterone was affected by brood size (LMM, F1,32 = 2.19, p = 0.15; F1,32 = 0.62, p = 0.44, respectively).

We tested whether any mismatch between begging and provisioning that resulted from cross-fostering affected the yolk testosterone deposition of the subsequent clutch. For this we applied a linear mixed model with either the yolk testosterone concentration or the total amount of yolk testosterone (see Supplementary material) as the response variable (n = 34 nests, 67 eggs). The difference in mean begging between foster and biological nestlings, egg position (=1st or 3rd egg), foster brood size, clutch size (= of the second clutch), and couple identity (= random effect) were included as explanatory variables. We included the interaction between the difference in begging and foster brood size, as the effect of a behavioral mismatch may become amplified with foster brood size.

All reported statistical tests were two-tailed and executed in R, version 2.10.1 (R Development Core Team, 2009). Extreme values (average ± 3 × standard deviation) were discarded from the analysis (Experiment 1: three values; Experiment 2: one value). The alpha value was 0.05. We present data as mean ± standard error. In addition, we provide the size of the effect of food provisioning on either the concentration or the total amount of testosterone, for both experiments. This was estimated by using the following formula: $\delta^2 = 1 - \text{variance residuals fixed (provisioning + the other factors) + random effect / variance residuals fixed (the other factors) + random effect}$.
3.2. Experiment 2: Allocation of yolk testosterone in response to previously experienced offspring demand and parental investment

3.2.1. Parental provisioning
The provisioning of both parents to foster nestlings in the previous clutch did not affect the yolk testosterone concentrations of the subsequent clutch ($F_{1,31} = 1.08, p = 0.31, \Omega^2 = 0.003$) (Fig. 3). Likewise, there was no statistically significant effect of egg position on yolk testosterone concentrations ($F_{1,32} = 3.63, p = 0.07$). However, clutch size had a significant negative effect on yolk testosterone concentrations ($F_{1,31} = 6.61, p = 0.01$).

The provisioning of each of the parents did not affect the yolk testosterone concentrations of the subsequent clutch either (maternal provisioning: $F_{1,31} = 0.61, p = 0.44, \Omega^2 = 0.002$; paternal provisioning: $F_{1,31} = 0.42, p = 0.52, \Omega^2 = 0.001$). The effects of the other factors were similar to those reported above, with the exception of egg position for the model of paternal provisioning which had a marginally non-significant effect ($F_{1,32} = 3.54, p = 0.07$).

3.2.2. Foster chick begging and growth
Yolk testosterone concentrations were not affected by the begging demands of foster nestlings in the previous clutch ($F_{1,29} = 0.00, p = 0.99$) (Fig. 4); this finding was independent of brood size (interaction begging x brood size: $F_{1,29} = 0.42, p = 0.52$). Neither foster brood size nor egg position had an effect on yolk testosterone concentrations (brood size: $F_{1,29} = 2.48, p = 0.13$; egg position: $F_{1,32} = 3.51, p = 0.07$). However, clutch size had a significant negative effect ($F_{1,29} = 6.85, p = 0.014$).

Growth rate of foster nestlings did not affect the yolk testosterone concentrations ($F_{1,31} = 1.01, p = 0.32$). The effects of the other factors were similar to those reported above.

3.2.3. Consequences of altered offspring demand
The difference in begging between foster- and biological offspring did not have an effect on the yolk testosterone concentrations of the subsequent clutch ($F_{1,29} = 1.65, p = 0.21$) (Fig. 5); this was independent of the brood size of the past clutch (interaction difference in begging x brood size: $F_{1,29} = 0.85, p = 0.36$). The effects of the other factors were similar to those reported in 3.2.2.

4. Discussion
Maternal yolk hormones are thought to facilitate the adjustment of the offspring’s phenotype to the environmental conditions it will experience post-hatching (Groothuis et al., 2005). Given that this early-life
Fig. 5. Differences in begging intensity between foster- and biological offspring, and the consequences of this mismatch for maternal yolk testosterone deposition in the subsequent clutch. The mean yolk testosterone concentration (of the 1st and 3rd egg) is plotted against the difference in mean begging intensity between foster- and biological nestlings. Negative values of begging intensity indicate that foster nestlings begged less than biological nestlings, and vice versa for positive values.

The levels of parental provisioning have been shown to covary with offspring begging in several species, including canaries (Kölliker et al., 2000; Agrawal et al., 2001; Hager and Johnstone, 2003; Lock et al., 2004, 2007; Hinde et al., 2009, 2010; Estramil et al., 2013, 2015). Such correlations may be genetic, as predicted by quantitative genetic models (Wolf and Brodie, 1998; Kölliker et al., 2005), or else may be driven by maternal effects such as maternally-derived yolk androgens (Hinde et al., 2009; Estramil et al., 2014a). The latter has been suggested based on studies in canaries, showing that (a) higher food quality before and during egg laying led to higher fecal androgen levels in both mothers and their offspring (Hinde et al., 2009), and (b) fecal androgen levels, in turn, were positively correlated with both parental provisioning (Hinde et al., 2009) and offspring begging (Buchanan et al., 2007).

However, this idea was not supported by our study, as there was no correlation between yolk testosterone levels and the provisioning of either or both parents when raising foster nestlings.

In this study, we did not measure parental or nestling hormone levels; thus, the mechanism(s) underlying the similarities in hormone levels across generations that has been previously reported (Buchanan et al., 2007; Hinde et al., 2009) are yet to be revealed. However, the lack of significant correlations between female provisioning and testosterone deposition suggests that females do not adjust offspring phenotype to their own ability to provide care. Likewise, they do not adjust offspring phenotype to their partner’s parental quality, since there were no significant correlations with respect to male provisioning. This result is in line with a number of previous studies (Bolund et al., 2009; Ruuskanen et al., 2009; Laaksonen et al., 2011).

The fact that yolk testosterone does not match parent and offspring behaviors is further supported by a recent study showing that a food manipulation, which indicates the food availability post-hatching, did not change clutch levels of yolk testosterone; however, it did affect the increase in yolk testosterone across the laying sequence (Vergauwen et al., 2012). This increase has been interpreted as a compensatory mechanism for hatching asynchrony, mitigating the disadvantages for later-hatching nestlings through positive effects on their growth and begging (Mock and Parker, 1997; Eising et al., 2001; Groothuis et al., 2005). Thus, females adjust offspring phenotype according to the food conditions within broods, but not among broods. One may also expect yolk testosterone deposition to vary with female body mass, as this is another factor that can affect the parental capacities to provide care. However, this was not the case in our study, and empirical evidence from studies testing for a relationship between maternal condition and the level of yolk androgens deposited is not consistent either (in canaries: Groothuis et al., 2005; Müller et al., 2012).

Taken together, the lack of a relationship between maternal yolk testosterone deposition and parental provisioning renders it unlikely that a previously reported covariation between offspring begging and parental provisioning (Hinde et al., 2009; Estramil et al., 2013, 2015) is mediated by yolk testosterone. Other maternal effects such as maternal yolk carotenoids may play a role (Helfenstein et al., 2008), but current evidence is limited. Ruling out maternal effects altogether, parent-offspring coadaptation may reflect an underlying genetic correlation, as predicted by quantitative genetic models (Wolf and Brodie, 1998; Kölliker et al., 2005). However, this requires more explicit investigation (but see Hager and Johnstone, 2003; Curley et al., 2004).

4.2. Retrospective allocation of yolk testosterone in response to previous offspring demand

Life-history theory predicts that parental investment in current reproduction will influence future reproduction due to its associated costs (Williams, 1966), which is supported by empirical studies showing that parental provisioning can affect future reproductive investment (e.g. Gustafsson and Sutherland, 1988; Field et al., 2007; Ward et al., 2009; Mariette et al., 2011). Therefore, we hypothesized that experimental changes in offspring demand, as achieved via full brood cross-fostering, affect parental investment in the subsequent brood (Hinde et al., 2010). There was no evidence from our experiment that this was the case. As in the previous experiment, females did not deposit yolk testosterone in response to partner quality, even though they were paired with the same male and thus possessed information on their parental qualities.

We additionally investigated whether changes in demand, on account of parents raising foster nestlings, impinged on yolk testosterone deposition of the subsequent clutch. However, we did not find any effect despite the fact that such carry-over effects have been described for other aspects of reproductive investment (Hinde et al., 2010). One possible explanation is that the costs of a behavioral mismatch are too small to influence subsequent yolk hormone deposition. In fact, in a recent cross-fostering study we showed that the provisioning of both parents was not affected by a change in the begging intensity of offspring (Estramil et al., 2014b). Furthermore, previous work on this species suggests that the costs of mismatching may be carried predominantly by the offspring (Hinde et al., 2010). Alternatively, it is possible that yolk hormone deposition does not serve as a means of offspring adjustment to parental provisioning, as supported by the results of Experiment 1 (this study).

Unfortunately, we do not have information about the yolk testosterone levels of the first clutch, which would have enhanced the statistical power by testing intra-individual changes, given the high level of heritable inter-individual variation (Tschirren et al., 2009; Okuliarova et al., 2011; Müller et al., 2012).
5. Conclusions

We did not find significant evidence for maternal yolk testosterone being deposited in anticipation of prospective parental provisioning, or in retrospect in accordance with previous parental investment. This suggests that in canaries, the correlation between offspring begging and parental provisioning is unlikely to be driven by yolk testosterone. Still, it could be the result of other (pre-natal) maternal effects or indeed reflect an underlying genetic correlation, which we cannot identify here. However, the level of yolk testosterone within clutches strongly increased with egg laying order, particularly in terms of the amount of testosterone deposited; supporting the role of yolk testosterone as a compensatory mechanism for hatching asynchrony.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.ybeh.2016.11.005.

References


