Maternal yolk testosterone in canary eggs: toward a better understanding of mechanisms and function

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Maternal yolk androgens in avian eggs have been shown to affect numerous offspring traits. These changes in offspring phenotype represent examples of maternal effects and are thought to adjust offspring development to the posthatching environment. When studying the functional consequences of yolk hormones it is, therefore, crucial to manipulate the yolk androgen concentrations as well as the environmental conditions under which the study is performed. However, so far context-dependent effects of maternal yolk hormones have not sufficiently been taken into account, which might contribute to the current level of inconsistency in yolk androgen-mediated effects. We experimentally elevated the yolk testosterone concentrations and manipulated the sibling size hierarchy. We focused on the effects of yolk testosterone on growth and monitored begging behavior and parental feeding preferences in search of the underlying mechanisms of changes in growth. Experimental changes in the yolk testosterone concentrations significantly affected offspring phenotype. However, elevated yolk testosterone concentrations only improved the growth of chicks that were at a competitive disadvantage, whereas it benefited all chicks when placed in staged competition for food as juvenile. This emphasizes the compensatory role of yolk androgens in the context of hatching asynchrony and its context dependency. Enhanced growth did not coincide with intensified begging. Neither males nor females preferentially fed chicks hatching from eggs with elevated yolk testosterone concentrations. Enhanced growth rather resulted from yolk testosterone induced changes in physiology, of which the detailed mechanisms are yet unknown. Key words: begging behavior, hatching asynchrony, maternal effects, sexual conflict. [Behav Ecol 21:493–500 (2010)]
context should also manipulate the sibling hierarchy, an approach that has rarely been taken (but see Eising et al. 2001). Furthermore, it remains unclear how yolk androgens modulate postnatal growth (Groothuis, Müller, et al. 2005; Gil 2008). One obvious suggestion is that yolk androgens stimulate begging, which results in a better access to food and leads thus to enhanced growth (Schwabl 1996). However, detected growth differences in relation to an experimental elevation of the yolk androgen concentrations do not always coincide with differences in begging behavior and vice versa (e.g., Eising and Groothuis 2003; Pilz et al. 2004). Other processes such as a yolk androgen-mediated changes the efficiency of nutrient ingestion (McCormick 1999), reallocation of resources (Groothuis, Eising, et al. 2005), or modifications of the metabolic system may also be involved (Tobler et al. 2007, but see Eising et al. 2001).

Finally, the resources that enable (yolk androgen mediated) enhanced growth—in most avian species studied so far—have to be provided by the parents. In this context, it has recently been hypothesized that this additional amount of effort differs between male and female parents. Females may via the extra resource provided by yolk androgens have on begging or growth—try to increase the male’s feeding effort whereas themselves being less responsive to these specific begging signals (reviewed in Moreno-Rueda 2007; Müller, Lessells, et al. 2007). Previous studies have indeed reported that females and males responded differently to different begging components, which in turn may be differentially modulated by yolk androgens (reviewed in Müller, Lessells, et al. 2007). However, 2 recent studies failed to show a change in parental feeding effort when the yolk androgen concentrations of the whole clutch were manipulated (Tscharirn and Richner 2008; Runskanen et al. 2009). Yet, these studies did not investigate possible within-brood feeding preferences in relation to the individual embryonic androgen exposure, which is of importance in a within-clutch context.

To investigate the context-dependent effects of yolk testosterone, we experimentally elevated the yolk testosterone concentrations whereas at the same time manipulating the position of the chick in the sibling size hierarchy (=context). We monitored changes in begging behavior and parental feeding preference in search of the underlying mechanisms of a yolk testosterone-mediated growth enhancement. We additionally studied the effects of yolk testosterone on the ability to obtain and defend a food source after a short food deprivation as juvenile. Here, all birds were—in contrast to the first part of the study—tested in the same context, in which it has previously been shown that birds benefit from elevated yolk androgen concentrations (Schwabl 1993; Strasser and Schwabl 2004; Eising et al. 2006, but see Müller et al. 2008).

We hypothesize that an elevation of the yolk testosterone levels would induce the same (organizational) changes of off-spring phenotype (Carere and Balthazart 2007; Groothuis and Schwabl 2008) but that the functional consequences vary with the context. Thus, as pointed out above, elevated yolk testosterone levels should benefit growth of chicks that are placed at a competitive disadvantage but not the growth of chicks in a competitive advantage. However, all chicks hatching from eggs with elevated yolk testosterone levels should have an improved ability to obtain and defend a food source. If the effects of elevated yolk testosterone levels on social dominance depend on the position in the sibling hierarchy, it is more likely that they represent rather an indirect consequence of (yolk androgen mediated) changes in growth or behavior during early development than an organizational effect (Carere and Balthazart 2007; Groothuis and Schwabl 2008). Thus, studying the long-term effects will not only improve our understanding of context dependency but also of the underlying mechanisms of hormone-mediated effects.

MATERIALS AND METHODS

Hormone manipulation and early development

At the beginning of March 2008, 240 Fife Fancy Canaries (120 males, 120 females) from the local breeding population were moved from the outdoor to the indoor aviaries and housed under a L:D regime of 15:9. Birds were provided with canary seed mixture (van Camp, Belgium), water, shell grit, and cuttlefish bone ad libitum. We provided the birds with egg food (van Camp) twice a week and daily after the chicks had hatched. After 5 weeks, 120 breeding pairs were formed, which were housed in separate breeding cages equipped with nest-boxes and nesting material. Yolk hormone concentrations of the first and second laid egg were manipulated 2 days after the first egg was laid (all other eggs were part of a different experiment). We injected either 50 ng testosterone dissolved in 5-μl sesame oil in order to elevate the concentrations of the first laid eggs to the levels of later laid eggs (first/second egg: 45.01 ± 26.57 ng/yolk, N = 14, third/fourth egg: 68.09 ± 44.16 ng/yolk, N = 11, both mean ± standard deviation, unpublished data from this population) or 5-μl sesame oil only as control. Both eggs within a clutch received the same treatment. There was no difference in the hatching success between the 2 treatments (N = 118 clutches, 64 vs. 65%, χ² test, P = 0.98).

Eggs were illuminated from beneath to check for development one week after injection. All eggs containing an embryo were cross-fostered placing 4 eggs of the same treatment and the same laying position in one nest to guarantee the identification of the treatment and hatching position. At the expected time of hatching, we monitored the nests 3 times a day in order to identify which egg a hatching had hatched from. Hatchlings were weighed to the nearest 0.01 g and marked with a nontoxic pen for individual recognition. To mimic hatching asynchrony, we placed 2 heavier and older chicks (“seniors,” one chick hatching from a testosterone-treated egg and one chick hatching from a control-treated egg, if possible of the same laying position) together with 2 younger and lighter chicks (“juniors,” one chick hatching from a testosterone-treated egg and one chick hatching from a control-treated egg, if of the same laying position) in an experimental nest. In total, we created 23 experimental nests. The juniors were on average 0.39 ± 0.12 days younger and 0.39 ± 0.03 g lighter (for detailed statistics, see below). The differences between chicks within their age category were 0.04 ± 0.07 g and 0.0 ± 0.0 days in case of the juniors and 0.09 ± 0.02 g and 0.09 ± 0.06 days for the seniors.

Thereafter, we measured the body mass (daily), tarsus length (on day 5, 8, 11, and 14), and the width of the beak (on day 5, 8, 11, 14, 17, and 20) of the chicks in the morning. Molecular sex determination (Griffiths et al. 1998) was used to determine offspring sex using a blood sample taken around fledging. There was no nesting mortality.

Nestling begging and parental feeding behavior

We measured begging within 1 h after hatching in those cases where the timing of hatching was exactly known following the protocol as established by Schwabl (1996). To this end, the chick was placed in an artificial nest. After 2 min of habituation, the nest was tapped 5 times within 1 min, and the number and duration of begging bouts was recorded. However, we only tested a subsample of chicks (N = 42) because it turned out that the number of chicks responding was low (see below). Begging was additionally measured daily until day 10 posthatching according to the protocol as described by von

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Engelhardt et al. (2006). Briefly, experimental nests were removed from their cages immediately after the lights were switched on and were taken into a room that was maintained at 30 °C. All chicks of an experimental nest were tested at the same time. To this end, chicks were placed into 4 visually separated artificial nests. Begging was stimulated by tapping with a metal rod against the beak whereas unaware of the chick’s treatment or category. We quantified the duration of begging and the begging posture in ascending order of vigor (0 = not begging, 1 = gape open, 2 = gape open, head back, 3 = as 2 plus neck stretched, 4 = as 3 plus back vertical, see Kilner 2002). The procedure was repeated 3 times, with an interval of 2 min. There was a sharp decrease in both begging time and begging postures after day 6 in the begging trials. This may be due to habituation, or to an increase in the fear response, because the eyes of the canary chicks are open from approximately days 4–5 (Bischof and Lassek 1985). Therefore, we restricted our analyses on the begging behavior to the first 6 days after hatching, in order to obtain a more accurate estimate of begging.

We measured parental feeding behavior in a standardized way where the chicks were 8, 9, and 10 days old. Chicks were marked on their head and subsequently fed with Ornix handmix (Orlux, Wielsbeke-Ooigem, Belgium) until satiation. To prevent parental feeding, all food was subsequently removed from the cage and the camera was placed in front of the cage for habituation. After 1 h, we returned the food and videotaped the behavior for ½ h. From the recordings, we selected for each parent the first recording that fulfilled the following criteria: 1) the parent of interest was feeding first to exclude effects of previous visits by the other parent and 2) all chicks were begging. It appeared to be difficult to obtain recordings of the male feeding first. Hence, we also videotaped the behavior of the male after we had separated the female from the cage (within sight) when the food was returned. In 17 broods, the recording fulfilled the criteria for the father and in 13 of those broods also for the female. A dip of the parent’s bill into the chick’s beak was scored as one food transfer, and we counted the number of food transfers during a feeding bout for each offspring.

We measured the begging behavior of the chicks during the feeding bout (feeding bout = arrival at the nest until the end of begging activity) by scoring the begging posture (every second, for posture details see above). We then summed the begging scores for each chick over the entire begging bout (Kilner 2002).

Juvenile behavioral measures

At approximately 7 months of age, we measured social dominance. We established pairs of birds of similar body mass, same category, same sex, not related, and that had received different in ovo treatment (N = 26 pairs). After a period of food deprivation (one night and an additional 6 h), a feeder with seeds was provided and the birds’ behavior was videotaped for 10 min. The videotapes were analyzed whereas unaware of the treatment the birds received. We measured the “number of wins” (sum of: the number of successful defenses of the food source when the other bird attempted to gain access, the number of successful takeovers of the food source, and the number of times a bird won in an overt conflict) and the time spent at the feeder (Müller et al. 2008).

Statistical analyses

We used hierarchical linear models to take the nested relationships and repeated measurements of the same individual into account (Snijders and Bosker 1999). Nonsignificant interactions and main effects were removed successively from the full model in a stepwise backward approach, starting with the least significant highest interactions, whereas the amount of data used in the compared models remained the same. Nonsignificant main effects were reintroduced into the minimum model. Only variables with P ≤ 0.1 were retained in the final model. Significance was tested using the increase in deviance (Δ deviance) when a factor was removed from the model, which follows a χ² distribution. Analyses were performed with MLwiN 2.0 (Rasbash et al. 2004).

“Body mass,” “tarsus length,” “beak width,” and “begging” were analyzed in a 3-level model: 1) foster nest, 2) individual offspring, and 3) repeated measure. We tested the effects of treatment (control = 0, testosterone = 1), offspring sex (female = 0, male = 1), category (junior = 0, senior = 1), age, and all interactions. To model the growth curve, we included the square of age and the cube of age as predictors in the models analyzing body mass and beak width, the square of age was included in the analysis of tarsus length (see, e.g., von Engelhardt et al. 2006). “Feeding behavior,” “begging postures during the feeding bout,” and “feeding distances” were analyzed in a 3-level model: 1) foster nest, 2) individual offspring, and 3) repeated measure. We tested the effects of treatment, offspring sex, category and parental sex (mother = 0, father = 1), and all interactions.

“Social dominance” (number of wins, “time spent at the feeder”) was analyzed in a 2-level model: 1) opponent pair and 2) individual. In the case of the number of wins, we used negative binomial model fitting because the data set contained a high number of zeros. We tested the effects of sex, treatment, category, and their interactions.

The data set has a cross-classified structure (genetic siblings and foster siblings), as in some cases both chicks of a given female were included in the experiment. When necessary we additionally used cross-classified models to take this into account; however, this made qualitatively no difference to the results. All other analyses were performed in SPSS 14.0.

RESULTS

Early development

There was a significant difference in body mass at cross-fostering between seniors and juniors (estimate = 0.39, error = 0.02, deviance = 117.31, P < 0.0001), but there was no difference between chicks hatching from testosterone-treated eggs (T-chicks) and chicks hatching from control-treated eggs (C-chicks; estimate = 0.003, error = 0.022, Δ deviance = 0.014, P = 0.91). There was also no interaction effect between treatment and age category (estimate = −0.01, error = 0.05, Δ deviance = 0.10, P = 0.76).

The subsequent “body mass gain” was different for T-chicks and C-chicks in interaction with sex and category (Table 1, Figure 1a). Separate analyses of both categories revealed that T-chicks grew better than C-chicks among the juniors, independent of their sex (treatment × age, estimate = 0.06, error = 0.01, Δ deviance = 15.46, P < 0.001, Figure 1a). In contrast, the effect of testosterone on the growth of the seniors was sex specific (treatment × sex × age, estimate = −0.05, error = 0.02, Δ deviance = 7.79, P = 0.005). Among senior males, G-males grew better than T-males (treatment × age, estimate = −0.02, error = −0.04, Δ deviance = 5.72, P = 0.02). Among senior females, there was no effect of testosterone on growth (treatment × age, estimate = 0.02, error = 0.01, Δ deviance = 2.31, P = 0.13).

The effect of testosterone on “growth of the tarsus” tended to be different between the 2 categories (Table 1). Testosterone enhanced the growth of the tarsus of the juniors (treatment, estimate = 0.55, error = 0.25, Δ deviance = 4.36, P = 0.04, Figure 1b) but not of the seniors (treatment, estimate = −0.17, error = 0.17, Δ deviance = 0.98, P = 0.32, Figure 1b). Male chicks had larger tarsi than female chicks (Table 1).
Table 1
Hierarchical linear model analysis of offspring development

<table>
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<tr>
<th>Factors</th>
<th>Estimate</th>
<th>Error</th>
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<td>0.22</td>
<td>4.4</td>
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</table>

There was an interaction effect of treatment on beak width with sex and category (Table 1). Testosterone had no effect on the beak width of seniors (treatment, estimate = -0.03, error = 0.07, Δ deviance = 0.23, P = 0.63) but a sex-specific effect on beak width of the juniors (treatment × sex, estimate = -0.39, error = 0.16, Δ deviance = 5.42, P = 0.02). Junior T-females developed larger beaks than C-females (treatment, estimate = 0.36, error = 0.11, Δ deviance = 8.45, P = 0.004), whereas junior T-males and C-males did not differ (treatment, estimate = -0.04, error = 0.12, Δ deviance = 0.11, P = 0.74).

Nestling begging and parental feeding behavior

**Begging within 1-h posthatching**

We measured early begging behavior for a subset of birds (27 C-chicks, 15 T-chicks), but there was no difference in the proportion of chicks that begged after stimulation (12 of 27 C-chicks, respectively, 6 of 15 T-chicks, χ² = 0.08, P = 0.78). There was no difference in the total begging duration (N = 18, T-chicks: 13.41 ± 3.52 s, C-chicks: 11.43 ± 2.25) or the number of begging bouts (N = 18, T-chicks: 2.33 ± 0.33, C-chicks: 2.08 ± 0.36) among the responding chicks (P > 0.63 in both cases).

**Artificial begging trials (days 2–6)**

The change in begging times with age was different for male and female chicks but was unaffected by treatment or category (sex × age, estimate = 0.77, error = 0.37, Δ deviance = 4.36, P = 0.04). Begging time for females but not male chicks tended to decrease with age (females: age, estimate = -0.43, error = 0.26, Δ deviance = 2.74, P < 0.1; males: age, estimate = 0.29, error = 0.26, Δ deviance = 1.22, P = 0.27). Chicks belonging to the juniors had a higher “begging score” than seniors (category, estimate = -0.82, error = 0.37, Δ deviance = 5.01, P = 0.05).

**Begging behavior during the feeding bouts (between days 8 and 10)**

Begging scores were significantly different between seniors and juniors, with older chicks having a higher score (category, estimate = 10.32, error = 3.54, Δ deviance = 8.19, P = 0.004, Figure 2) but unaffected by yolk androgens (treatment, estimate = -1.36, error = 3.52, Δ deviance = 0.15, P = 0.70). The begging scores were higher when the male was feeding compared with the times the female was feeding (parental sex, estimate = 7.58, error = 3.85, Δ deviance = 3.85, P < 0.05, Figure 2).

**Parental behavior**

The number of food transfers made by the parents was different between seniors and juniors and tended to depend on the sex of the parent (category × parental sex, estimate = 2.97, error = 1.65, Δ deviance = 3.12, P = 0.08) but did not differ between T-chicks and C-chicks (treatment, estimate = 0.86, error = 0.87, Δ deviance = 0.96, P = 0.33). Mothers preferred seniors (category, estimate = 2.83, error = 0.95, Δ deviance = 8.06, P = 0.005, Figure 3a) and tended to make more food transfers to male chicks (offspring sex, estimate = 1.83, error = 0.98, Δ deviance = 3.19, P = 0.07). Fathers had a strong preference for seniors (category, estimate = 5.61, error = 1.33, Δ deviance = 15.90, P < 0.0001, Figure 3b). Whether the female was within or next to the cage did not affect male feeding behavior (estimate = 0.69, error = 1.41, Δ deviance = 0.24, P = 0.65).

We compared the feeding behavior of the parents in those 13 cases where we did record both partners. There was no difference in the number of food transfers between males and females in a within-pair comparison (paired t-test, t = -1.25, P = 0.24), but males tended to have longer feeding bouts (paired t-test, t = -2.18, P = 0.05).

**Behavioral measures of the juveniles**

As juveniles, testosterone birds (T-birds) spent significantly more time at the feeder than control birds (C-birds) (estimate = 102.79, error = 34.19, Δ deviance = 8.38, P = 0.004, Figure 4a). T-birds were also significantly more aggressive than C-birds (estimate = 1.05, error = 0.44, χ² = 5.69, P = 0.009, Figure 4b). The level of aggression was higher in male–male encounters (sex, estimate = 1.72, error = 0.45, χ² = 14.61, P = 0.0001). The effect of treatment was in both cases not different between juniors and seniors (time spent at the feeder: Δ deviance = 1.46, P = 0.29; aggression: Δ deviance = 0.59, P = 0.44). There were no long-lasting effects on body mass (P > 0.17 in all cases).

**DISCUSSION**

**Yolk androgens as mediator of sibling competition**

There are an increasing number of studies that failed to confirm the earliest findings of yolk androgens enhancing begging and growth (e.g., Schwabl 1996; Eising and Groothuis 2003; von Engelhardt et al. 2006; Sockman et al. 2008). At present it is, therefore, difficult to generalize whether yolk androgens indeed form a mechanism for the mother to mediate sibling
rivalry as has originally been hypothesized (Schwabl 1993, 1996; Eising et al. 2001). Some of the inconsistency may relate to methodological issues such as the amount of androgens injected (Navara et al. 2005; Cucco et al. 2008). However, as means of a maternal effect yolk androgens are thought to adjust offspring development to given environmental conditions (Mousseau and Fox 1998). The functional consequence of maternal yolk androgens will, therefore, probably vary with the within-brood conditions that are strongly affected by the hatching position. Indeed, here, we clearly show that junior chicks that are placed at a competitive disadvantage similar to later-hatching chicks in natural broods benefited from elevated yolk testosterone levels in terms of enhanced growth (Figure 1). Although the effects on growth were only temporarily (no difference in body mass as juvenile; see also Schwabl 1996), small size differences during early development may have strong effects on competitiveness and survival if the food conditions are less favorable or with larger brood sizes.

This strongly supports the hypothesis that yolk androgens function as compensatory mechanism for delayed hatching (Schwabl 1993, 1996, reviewed in Groothuis, Müller, et al. 2005; Gil 2008). Interestingly, the functional consequences of yolk androgens were different among the senior chicks that were given an advantage in sibling competition, although the experimental elevation of the yolk testosterone concentration was identical. Here, male chicks even suffered from elevated yolk testosterone concentrations in terms of reduced body mass gain, whereas females were unaffected. This sex-specific effect suggests that embryonic exposure to maternal yolk androgens is not only unnecessary for senior chicks, which are probably growing at a maximum rate anyway, but also that senior males would benefit from lower yolk androgen levels. Similar sex-specific patterns have been found in a number of recent studies, but the underlying mechanism is as yet unknown (Müller et al. 2005, 2008; von Engelhardt et al. 2006; Sockman et al. 2008; Pitala et al. 2009, but see Saino et al. 2006). These studies also show that the strength of selection on yolk hormone levels will also depend on the magnitude of the effects in female offspring, which could also lead to sexually antagonistic selection. Furthermore, clearly, other environmental factors such as harsh food conditions or large brood sizes may change the consequences of yolk testosterone for senior chicks.

Contrasting effects of yolk androgens on offspring development within a study have previously not been demonstrated and our findings have, therefore, important implications for
likely that an experimental elevation of the yolk androgen concentrations induced different changes in the chick phenotype. Rather, the functional consequences as measured in terms of growth varied with the context as they depended on the experimentally manipulated position in the sibling size hierarchy. Indeed, birds hatched from testosterone-treated eggs were more successful at obtaining and defending a food source, when placed in an identical context (staged competition for food as juvenile; Figure 4; Schwabl 1993; Strasser and Schwabl 2004; Eising et al. 2006, but see Müller et al. 2008).

One possible mechanism could be that yolk testosterone influences several traits independently of each other (Groothuis and Schwabl 2008) and that resulting trade-offs between costs and benefits vary with the circumstances under which the study is performed. Thus, the environment determines what kind of effect will be measured, especially when studying phenotypic endpoints of different underlying yolk androgen induced physiological changes such as growth (Navara and Mendonca 2008).

To our knowledge, only one previous study has tested the adaptive significance of among-clutch variation also manipulating the environment in a similar way as we tested within-clutch variation in this study but failed to find an environmental influence on the effects of their yolk testosterone injection (Tschirren et al. 2005, for studies that integrated nonmanipulated variables from the environment in their analysis, see also Müller, Deputch, et al. 2007; Pitala et al. 2009). However, given the results from this study, it is very likely that the understanding of the adaptive significance of among-clutch variation in yolk androgens will strongly benefit from additional context-dependent experiments.

Yolk androgen-mediated growth enhancement—in search of mechanisms

We failed to detect any effect of yolk testosterone on begging behavior, which renders it unlikely that yolk testosterone-mediated begging represents the mechanism by which enhanced growth is achieved (Figure 3, Eising and Groothuis 2003; Pilz et al. 2004). At present, only 2 of 5 studies showed that the detected growth differences in relation to an experimental elevation of the yolk androgen concentrations coincide with differences in begging behavior (Schwabl 1996; von Engelhardt et al. 2006 vs. Eising and Groothuis 2003; Pilz et al. 2004, this study). In addition, no effects on begging have been found in a study that did not measure chick growth (Boncoraglio et al. 2006). It has been suggested that
Yolk androgens as mediator of sexual conflict over parental investment

The costs of raising chicks hatching from eggs with high androgen concentrations may not be equal for male and female parents. As recently suggested, maternal yolk androgens may offer a mechanism by which females exploit the male's contribution to parental care (Moreno-Rueda 2007; Müller, Lessells, et al. 2007). However, here, we show that there are no sex-specific feeding preferences, although the parents could freely choose within broods between offspring that differed in embryonic exposure to yolk testosterone (Figure 3). This suggests, together with the 2 previous studies that failed to detect an effect of experimentally manipulated yolk androgen concentrations on growth, that the females do not manipulate male feeding behavior by means of differential yolk androgen deposition or that males are able to resist such manipulation (Tschirren and Richner 2008; Ruuskanen et al. 2009, this study).

However, given the enhancing effects of yolk androgens on the growth of later-hatching chicks (see above, Figure 1), females may well synchronize their broods by means of differential yolk androgen deposition within the laying sequence. Thereby they may reduce their own parental effort at the cost of their mates, given that males contribute more to asynchronous broods in favor of a limited number of larger chicks. However, within the first feeding bout, males and females did not differ in the total amount of food transferred, but all chicks were hungry and it is still possible that males reduce their care as soon as the largest chicks are satiated.

CONCLUSIONS

The results of our study strongly support the original hypothesis that an increase in yolk androgen concentrations with laying order functions as a compensatory mechanism for delayed hatching (Schwabl 1993, 1996; Eising et al. 2001). With this study, we also show, for the first time, that the functional consequences of yolk androgens differ with the (within-brood) context. This highlights the importance of our experimental approach that included a manipulation of the environmental conditions in order to improve our understanding of the adaptive significance of yolk hormone-mediated maternal effects. Care has to be taken when interpreting the effects of yolk androgens based on studies addressing the functional consequences in terms of phenotypic endpoints such as growth. Differences in growth probably reflect the integration of many different physiological changes of which the knowledge of how they are affected by yolk androgens is yet very limited.

The effects of elevated yolk testosterone on the growth of the chicks were independent from changes in begging behavior and/or parental feeding preferences. This reduces the probability that yolk androgen-mediated higher growth rates are mainly achieved through enhanced begging or better access to food but rather by as yet unknown physiological changes. The lack of a positive effect of yolk androgens on begging also reduces the possible pathways available to the female to manipulate the male's feeding behavior. Accordingly, males did not preferentially feed chicks hatching from eggs with elevated yolk testosterone concentrations in a within-brood context.

FUNDING

Research Foundation Flanders (FWO) postdoctoral grant (G.0130.07 to M.E.); and a FWO research project (G.0130.07 to M.E.).

The experiment was performed under proper legislation of Belgian and Flemish laws. We thank Jonas Vergauwen and Tom Smet for their support with the practical aspects of the experiment.

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