Maternal hormones meet environmental variability
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MATERNAL HORMONES MEET ENVIRONMENTAL VARIABILITY
Context-dependent effects of maternal hormones in avian egg yolks
English Summary

Over the past 2 decades, maternal effects have been recognized as an important pathway through which maternal phenotypes can modify offspring phenotype over and above direct genetic pathways. In very many animal taxa, embryos are prenatally exposed to maternally-derived hormones. This has led to a general acknowledgement that maternal hormones serve one of the major avenues for maternal effects to take place, which has been widely supported by empirical data. In birds, the research of maternal hormones has experienced a period of rapid growth since Schwabl’s pioneering study that reported the potential fitness consequences of yolk androgens of maternal origin in avian eggs. Nevertheless, the adaptive significance of prenatal maternal hormone exposure is still elusive because of the large discrepancy among studies. This situation seems to have lowered the interest of researchers and impedes the further advancement in this field. A promising solution is that many environmental or biological contexts may have great potential to modulate the final outcomes of prenatal androgen exposure, leading to inconsistencies in the literature. This is theoretically very likely as maternal hormones may not only bring benefits but also costs to the offspring. In this thesis, taking the most-extensively studied maternal hormones – testosterone (T) – as an example, I first explained the theoretical basis of why the effects of maternal androgens may be context-dependent and when we can expect to see context-dependent effects (Chapter 1). Second, I extensively reviewed the literature, discussed relevant contextual cues, and proposed possible experimental designs (Chapter 1), in order to stimulate further studies.

Being exposed to elevated levels of maternal T may boost the development and growth of the offspring and enhance their competitiveness and aggression. However, some costs are also incurred aside from these beneficial effects, for instance, elevated metabolic rates and thus higher energy expenditure, suppressed immune responses, and altered status of balance between oxidative damages and anti-oxidant capacity. Therefore, any contexts that can alter the cost-benefit balance might be able to modulate the final outcome of prenatal exposure to maternal hormones. In order to provide empirical data, I experimentally tested the context-dependent effects of maternal yolk testosterone, using the rock pigeons (*Columba livia*) as the subject. In these serial experiments, the modulation of three contextual cues – food conditions, offspring age, and egg composition – on the effects of yolk T were investigated. In chapter 2 and 3, the data concerning food conditions were presented. In chapter 4 and 5, as follow-ups of chapter 3, offspring age was taken into account to test the long-lasting effects prenatal T exposure and early-life food restriction respectively. More importantly, I also analysed whether or not these effects change over age. In chapter 6, based on the results of chapter 2, I manipulated yolk
thyroid hormones of pigeon eggs. The first goal of this experiment was to test the significance of yolk thyroid hormones on the offspring, which has been long overlooked in the literature. Secondly, by manipulating yolk thyroid hormones in eggs that naturally differ in androgen concentrations, I was allowed to test the potential interaction between yolk thyroid hormones and yolk androgens.

**Chapter 2: differential allocation of egg substances according to food conditions**

In chapter 2, the effect of food conditions before and during egg-laying on maternal hormone allocation was tested. In order to provide basic knowledge for testing the effects of differential egg composition, not only yolk androgens (T and A4, androstenedione), but also two thyroid hormones (T3, triiodothyronine, and T4, thyroxine) were measured. In this experiment, breeding pigeons were housed in either a food-enriched or a food-restricted condition before and during egg-laying. This species typically lays only two eggs per clutch. The second eggs (B-egg) were always found to contain higher levels of yolk T and A4 than the first eggs (A-eggs), independent of our food treatment. In contrast, yolk T3 concentrations were higher while the total amount of yolk T4 were lower in the eggs laid under the food-restricted conditions, but neither of them showed clear within-clutch difference. Egg mass and yolk mass showed both significant reductions under the food-restricted conditions. These results suggested that different components of egg substances were probably deposited via different mechanisms. For some components, the production and deposition largely depend on maternal nutrition (e.g. egg and yolk mass) or the availability of certain ingredients (e.g. iodine for thyroid hormones). In this case some constraints or trade-offs are very likely to influence maternal allocation into the eggs. In contrast, the deposition of other egg substances made of abundant precursors (e.g. androgens made from cholesterol), might be free of constraint or trade-off and there might be more freedom for maternal adjustments according to external cues. The fact that mothers did not strongly modify egg composition in relation to food may be due to the possibility that food availability at the time that chicks hatch is difficult to predict at the time of egg production. However, this may not be a problem for maternal fitness in case the effect of yolk T on the chicks is food dependent. This is explored in chapter 3.

**Chapter 3: food-dependent effects of yolk T**

In chapter 3, following chapter 2, I did an experiment with a two-by-two factorial design, combining yolk T manipulation and food treatment, to test whether elevated yolk T will have differential effects under different post-hatching food conditions. In this experiment,
only A-eggs, which contain lower levels of yolk T, were used so the potential within-clutch difference in egg composition over laying order cannot interfere with the results. Half of the eggs were injected with T solution, mimicking the higher average T levels in B-eggs. Hatched chicks from opposite yolk T treatments (T-injected versus control vehicle-injected) were further matched by the date of hatching, body mass at hatching, and their sex, in order to isolate the effects of yolk T and increase the sensitivity of the experiment. The results indicated that only in the food-restricted condition, T-chicks showed a significantly higher mortality than control chicks, whereas in the food-enriched condition, T-chicks grew better. These results strongly support the hypothesis that the effects of yolk T can be context-dependent. The underlying mechanism is as yet unclear, but a possibility is that under food restriction the T-chicks could not afford the elevated energy expenditure or lower immunity induced by elevated prenatal T.

**Chapter 4: long-lasting effects of yolk T into adulthood**

In chapter 4, the pigeons from three yolk T injected experiments in three consecutive years (from 2011-2013) were used to analyse the long-lasting effects of elevated yolk T. Apart from the long-lasting effects of yolk T, the potential age dependent effects of yolk T were also analysed. Despite that T is well-known to have organizational effects on brain and behaviour in adulthood, previous studies only provided data in the juvenile stage or the first breeding seasons. Behaviour, however, can be easily changed or masked by experience or learning over time. In this experiment, I analysed the reproductive behaviour of pigeons, including courtship, pair-bonding, and aggression, in a single breeding season (2014). Indeed elevated prenatal yolk T exposure showed long-lasting effects on both sexes. T-males were found to be less aggressive however similarly successful as C-males in reproduction. T-females were found to lay significantly lighter eggs than C-females. The pair-bonding combination among pigeons also showed a strong disassortative pattern with respect to yolk T treatment, with more than twice numbers of opposite-treated pairs (T-C or C-T) as same-treated pairs (T-T or C-C). More interestingly, these effects did not show any sign of attenuation over age. This indicates that for a complete picture of the function of yolk hormones both the chick and adult stages should be taken into account.

**Chapter 5: long-lasting effects of early-life food restriction**

In addition to prenatal avenues such as hormone exposure, maternal effects can take place via postnatal routes, for example, parental care. In 2012 and 2013, two experiments
were conducted with post-hatching food restriction. In 2012, food restriction was applied for the entire nestling period (from hatching to day 26), while in 2013 only from hatching to day 8. As the chicks in both experiments were age-matched, I thus was able to also test whether the severity of food restriction delivers different effects on reproductive behaviour in adulthood. The results showed that early-life food restriction significantly reduced survival and adult body mass, despite that remarkable compensatory growth was also observed. Furthermore, the courtship display of males, how often a female being courted, and pair-bonding behaviour in both sexes were all significantly reduced by early-life food restriction. For the former two, the more severe post-hatching food restriction (in 2012) showed stronger effects. There was also some evidence that the degree of compensatory growth also yielded negative effects in adulthood.

Chapter 6: effects of yolk thyroid hormones and their potential interaction with yolk testosterone

The data presented in chapter 2 clearly indicate that environmental context, such as food, can significantly influence egg composition. Making use of the natural difference of yolk androgens between A- and B-eggs of pigeons, other egg substances such as in this case thyroid hormones, can be manipulated to create contrasts on egg composition and study whether egg composition may modulate the effects of yolk androgens. To do this, I chose to manipulate yolk thyroid hormones (THs). First of all, unlike androgens, TH levels in the egg yolks of pigeons do not systematically vary across the laying order despite still showing considerable variation (Chapter 2). This provides an excellent opportunity to test the potential interaction between androgens and THs in egg yolks by only manipulating yolk TH levels. Second, despite that the presence of THs of maternal origin in avian egg yolks has been confirmed in early 1990s, the functional significance of these non-steroid hormones is almost completely overlooked. However, THs, given their essential role in embryonic development, are very likely to account for important variation in offspring phenotype. I thus raised yolk THs levels by 2 SD in both A- (with low levels of yolk androgens) and B-eggs (with high levels of yolk androgens) and followed the growth of nestlings until fledging. Yolk THs did not seem to interact with yolk androgens, except a non-significant trend of hatching acceleration by elevated yolk THs, apparently only in B-eggs, was observed. Nevertheless, the results showed interesting effects of yolk THs on nestling development, which warrants further studies. On the one hand, elevated yolk THs significantly enhanced the proportion of successful embryonic development and hatching, while on the other hand reduced nestling body mass from day 14 to around fledging (day 23). Furthermore, sex-specific effects on hatchling metabolic rates and circulating T4 levels
at posthatching day 14, with an increase in females but decrease in males, were also found. The mechanisms underlying these effects are still unclear, but these results should warrant further studies on maternal THs and open a new field in the study of maternal hormones.

Conclusion

In conclusion, the data presented in this thesis suggest that the effects of yolk T are likely to be context-dependent (Chapter 3), albeit not necessarily for all possible contexts. This suggests that the discrepancy in the literature might not be inconsistency, but reflect a more subtle and complicated scheme waiting for better understanding. Therefore, in order to bring this field to the next stage, it is important to take contexts into account in future studies. Given that studies on the modulation on yolk T effects under many other relevant contextual cues (see Chapter 1) are still lacking, we certainly need more studies to confirm how prevalent and under which contexts the effects of maternal hormones would be different. Egg yolks consist of many different substances. Aside from paying more efforts in understanding the function of the egg substances that are underexplored like yolk THs, applying more integrative approaches taking interactions among these different components into account is indispensable.