Neglected aspects of hormone mediated maternal effects

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Chapter 7

Summary, synthesis, and future perspectives

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This thesis investigated so far neglected aspects of hormone mediated maternal effects in birds. Vertebrate embryos are exposed to maternal hormones that can have consequences for the offspring phenotype, thus possibly mediating maternal effects (hormone mediated maternal effects). Egg laying species have been used extensively to study such effects as the embryo develops inside the egg, where the mother deposits her hormones, facilitating experimental manipulations of this exposure without interfering with the mother. Bird species are a particularly suitable model since bird ecology is often well-known and can easily be studied in the field, facilitating studies of the adaptive significance of maternal effects.

Several studies have injected steroids, mostly androgens, into bird eggs, mimicking variation in maternal yolk deposition, and found a wide array of effects of the treatment on the offspring. The phenotypic effects include traits such as hatching time, hatching success, metabolic rate, immune function, endocrine function, growth, competitiveness, reproduction, mate choice, and survival rate. This suggests that the mother is able to adjust the offspring phenotype adaptively to the current environment via her steroid hormones, but the underlying mechanisms are unknown, importantly (i) the maternal hormone allocation is often not determined correctly, and (ii) the embryo is often seen as a passive player and assumed to be a 'slave' to the maternal signals, but its actual role in translating maternal hormonal signals in proximate and ultimate manners (see Fig. 3 in **chapter 1**) is poorly understood. Below I summarize the main results of the different studies that I performed, how the different studies relate to each other, and future perspectives.

CHAPTER 2

The dose of steroid manipulation used in experimental studies is based on the natural levels, which are mostly measured by radioimmunoassays (RIA's). In **chapter 2** we addressed the question: are RIA-based hormone measurements in eggs reliable? We found that commonly used RIA method, i.e. without extensive extraction and purification steps as well as antibody characterization, gave much exaggerated hormone measures compared to a more reliable mass spectrometry method. Therefore, the findings from RIA-based experimental manipulation of egg hormones seem unreliable due to use of pharmacological doses instead of the intended manipulation in the physiologically occurring range. Further, as the extent to which RIA's gave exaggerated measures depends on the species as well as egg laying order for the same species, RIA-based studies also seem to be inadequate for interpretations of within species variations in maternal hormone allocation, as well as between-species comparisons, affecting the eco-evolutionary explanations for hormone mediated maternal effects.

Therefore, hormone mediated maternal effects should be verified using biologically relevant (i.e. within physiological range per species) hormone doses based on mass spectrometry measures. This could also potentially explain the discrepancy reported in the literature (some studies find a positive effect of hormone injections on a certain trait while others find no effect or negative effects) as the extent to which different studies injected pharmacological doses might differ per species and egg laying order, and the characteristics of the RIA used.

Furthermore, 5α-dihydrotestosterone (DHT), the most potent androgen (Fang 2003), is often reported to be present in the egg yolk in the studies based on RIA's (e.g. (Eising et al. 2008; Elf & Fivizzani 2002; Groothuis & Schwabl 2002; Schwabl 1997a)). Intriguingly, 5α-DHT was not even detectable in the egg yolk of any of the three bird species that we studied (black headed gull, rock pigeon, and red jungle fowl), when analysed by LC-MS/MS, in spite of its quantification limit being as low as 0.1 nmol/L, warranting another caution for the reliability of classical radioimmunoassays for hormone analyses of eggs.

CHAPTER 3

The differential amount of maternal hormones in the yolk of freshly laid eggs as per the maternal environment as well as the egg laying order is assumed to represent differential maternal allocation. Several studies have injected hormones in bird eggs mimicking this differential embryonic exposure to maternal hormones. However, this approach has revealed inconsistencies in the magnitude and direction of hormone mediated maternal effects (see above). We found that (1) maternal hormones in bird eggs are metabolized already surprisingly early, between ovulation and oviposition, (2) the rate and direction of metabolism differs between eggs that differ in their position within the laying order, and (3) this is not due to maternal enzymes in the yolk.

These findings have important consequences for the field of hormone mediated maternal effects: (1) since the estimates of maternal hormone allocation from oviposition for egg injections are inadequate, the interpretation of both the results of such experiments and their ecological and evolutionary interpretations requires validation, and (2) different eggs of the same species show systematic differences in hormone metabolism, potentially explaining discrepancy in the outcomes of hormone injection experiments as well as opening up new perspective on family conflict if this extremely early metabolism is embryonic.

CHAPTER 4

It is generally thought that maternal egg steroids must reach embryonic body tissues in order to be functional. But how and when this happens remains unclear, including whether maternal androgens reach embryonic tissues in their active form or are metabolized instead. We injected stable isotope labelled primary androgens, testosterone (T) and androstenedione (A4), into chicken egg yolks and tested their presence in embryonic tissues after five days of incubation. Using mass spectrometry, a highly reliable method for correct molecular identification, we found that injected androgens are almost completely cleared from the yolk-albumen homogenates but were not found in embryonic tissues, indicating their conversion to other substances. We also showed that these androgens are not conjugated or converted into a more potent downstream metabolite, DHT, or its conjugate. Furthermore, androgens are unlikely to be aromatized into estrogens, such as estradiol (E2), at such an early stage of embryonic development as aromatase (the enzyme necessary for aromatization) is expressed only after day 5 of incubation in chicken (Nomura et al. 1999), which is also experimentally supported by the lack of such early androgen conversion to estrogens (Parsons 1970).

Our findings indicate that egg androgens are converted into metabolites of which the biological functions are yet uncharacterized. These findings also suggest that the maternal effects of increased hormone exposure of the embryo are either mediated by the metabolites themselves, or the effects take place very early (before active hormones are metabolised), perhaps by receptor-mediated gene regulation, or by non-genomic pathways during early embryonic development. Thus, our findings open up new research avenues for mechanisms underlying hormone mediated maternal effects. Furthermore, the results may also explain how maternal gonadal steroids could exert their effects without interfering with sexual differentiation as they are metabolized even before the sexual differentiation of gonads and brain, and embryo's own hormone production starts.

CHAPTER 5

It is often assumed that in hormone mediated maternal effects the embryo only plays a passive role, but some studies indicate otherwise (Paitz & Casto 2012; Paitz et al. 2011; von Engelhardt et al. 2009). To study the role of the embryo, we analysed the dynamics of a wide range of gonadal steroid hormones over the early incubation phase (first 4.5 days) in rock pigeon eggs, where latter laid eggs of a clutch receive more maternal androgens. We found that (1) the active maternal hormones such as progesterone (P4) and T were substantially and rapidly metabolized, with a corresponding increase in supposedly less potent metabolites, such as pregnanolone and etiocholanolone, already within one-fourth of the total incubation period, (2) this only occurred in fertilized and not in unfertilized eggs,

and (3) the dynamics of androgen metabolism was different between eggs of different laying position – latter laid eggs had a higher androgen metabolism.

Our results not only confirm a few earlier studies on the role of the embryo in translating maternal hormonal signals, but also show that eggs differ systematically (higher metabolism in the latter laid eggs of a clutch) in the hormone conversion according to their position in the laying order within the clutch. This suggests that the embryo might be able to adjust its metabolic capacity according to maternal signals, as maternal hormone deposition differs over the laying order. This also suggests that embryos are active players in dealing with maternal signals instead of having a passive role, as is widely assumed, and thus may play their own important role in the family conflict. For instance, the mother might manipulate egg hormones in order to enhance her own fitness over her offspring's fitness depending on factors such as quality of the father and sex of the offspring, in combination with other environmental cues. Thus, embryos that can actively regulate their endocrine environment will be favoured by natural selection. Our results indicate that in studies of hormone mediated maternal effects the role of the embryo has not received the attention it deserves.

However, the results also create an apparent paradox, as it is well known that initial differences in these hormones can have substantial effects on the chick, whereas we show that at 4.5 days of incubation these differences are hardly present anymore. This paradox can be solved in three ways: first, the active hormones can already induce receptor-mediated genomic and/or non-genomic changes in embryonic tissues before their depletion; second, steroids may intercalate with DNA and thereby have nonreceptor mediated genomic effects; third, the embryo may convert the conjugated metabolites back to their unconjugated forms in the course of development, and these metabolites themselves might be biologically active and may act non-genomically (i.e. by affecting not directly the gene expression but other cell-signalling pathways). Alternatively, because the findings that initial egg differences in maternal hormones can have substantial effects on the chick development and behaviour were performed using pharmacological doses (see **chapter 2**) and measured at seemingly inappropriate time-points (see **chapter 3**), those findings themselves might be biologically irrelevant. These possibilities are promising avenues for further research.

The findings further solve a long-standing puzzle of how maternal gonadal steroids in the egg can induce maternal effects without interfering with embryonic sexual differentiation, as we show that the embryo is capable of metabolizing most of the active maternal steroids as early as even before the process of sexual differentiation starts. These findings are in our view important for guiding the mechanistic studies that are urgently needed for correct functional interpretations of hormone mediated maternal effects, at the same time opening-up new research possibilities from ecological perspectives as well.

CHAPTER 6

In order to be functional, the androgens must reach the embryonic tissues and those tissues must have androgen receptors (AR). However, very early in incubation, yolk androgens are substantially and rapidly metabolized to supposedly less potent forms by the embryo (see **chapters 4 and 5**). Moreover, the steroids are lipophilic, and how the embryo is able to take up these hormones from the yolk in its watery circulation to exert their effects on body tissues remains an enigma. Using chicken eggs, we discovered for the first time that the embryo expresses AR and estrogen receptors (ER) in its extra-embryonic membranes (EMs), already as early as one-fourth of the total egg incubation period until hatching, before its own hormone production starts, providing a novel pathway for action of maternal hormones. Lastly, we show that the embryo downregulates AR expression in the EMs with increased yolk androgen levels, showing that the embryo can adapt to maternal signals.

The results move the research field forwards by providing solution to three long-standing problems in the field: (1) the problem of hormone uptake from lipophilic yolk into embryonic circulation, since we show that the uptake might not be necessary – the hormones can act on the receptors of EMs, which are just at the interface of yolk and embryonic circulation, (2) the question whether the embryo is just a passive receiver of the mother's signals, giving the mother the upper hand in family conflict. As we show an active role of the embryo in adapting its receptor expression in the EMs to yolk hormone levels, it indicates that the embryo is not just a slave to maternal signals, and (3) maternal gonadal steroids can exert their effects without interfering with embryonic sexual differentiation as the hormones need not reach embryonic body tissues but can act via EMs. Such early and non-specific action of maternal hormones can also explain why their effects on the offspring are so diverse in nature, as the effects would take place very early during building of an organism, and hence broad, instead of being limited to a particular tissue, such as brain or gonads.

These findings open up a new avenue not only for other oviparous species but also for mammalian research, where the expression of steroid receptors in the fetal placenta nor their expression in relation to maternal hormones has been studied in such early phase. The results also open up new research directions concerning mechanisms for hormone mediated maternal effects, such as activation of the EM receptors and their downstream cellular and physiological effects in the embryo.

1 SYNTHESIS

1.1 Interrelations between the findings of different chapters

The main implications of my studies are listed below (A-F) and in Table 1 the evidence for these are listed per chapter.

Table 1. The key findings presented in this thesis and their potential implications.

Chapter	Key findings	Implications
2	(i) The levels of maternal steroids in the egg are actually much lower than previously thought. (ii) This difference is dependent on species and egg laying order.	A, B
3	The actual maternal allocation around the time of ovulation is different than found at oviposition, and the level of difference depends on egg laying order.	B
4	Yolk androgens are not taken up but metabolized substantially and rapidly by the embryo into supposedly much less potent metabolites.	C, D
5	(i) Same as (4) above. (ii) The extent of embryonic metabolism is higher in eggs which received higher maternal deposition.	C, D, E, F
6	(i) Maternal hormones may act via their receptors in the EMs and need not necessarily reach embryonic body tissues. (ii) The embryo can adjust its AR expression in the EMs in response to yolk androgen levels.	D, E, F

A. RIA-based hormone injection experiments in ovo are unreliable due to use of extremely high (pharmacological) hormone dose, the effects shall be verified using physiological dose. Also, RIA-based descriptive and comparative studies are unreliable, and thereby their eco-evolutionary explanation. This conclusion is based on the fact that such experiments are calibrated using RIA measurements of hormone levels in unmanipulated eggs whereas LC-MS/MS measurements indicate that these are substantially over-estimated (**chapter 2**). Though it could be argued that pharmacological doses may still induce normal effects due to a ceiling effect when all receptors are occupied by the hormone, but dose dependent effects have been demonstrated in many cases (Muriel et al. 2015; Podmokła et al. 2018; von Engelhardt & Groothuis 2011). Therefore, dose-response curves should be established in order to discern these possibilities.
B. Using hormone concentrations in the egg at oviposition is the golden standard for measuring maternal hormone allocation to her eggs. However, this is incorrect, as hormone concentrations differ between the time around ovulation (the time directly after the mother deposited the hormones in the yolk) and oviposition (**chapter 3**). This jeopardizes potentially many adaptive explanations as these may

be based on wrong data. Moreover, the hormone conversion in the oviduct differs among eggs (**chapter 3**), potentially explaining the discrepancy in the magnitude and direction of injection effects reported in the literature, as eggs of different laying order might have been injected with inappropriate doses.

C. Effects of increased hormone exposure of the embryo are either mediated very early, before conversion of active hormones has taken place (possibly via receptors in the EMs), or by the metabolites themselves, or perhaps by both in a temporally coordinated manner. This is because the free hormones are almost completely metabolized in the first 4 to 5 days after incubation (**chapter 4 and 5**). These possibilities can be discerned by examining the changes in embryonic physiology at several time points in response to injected steroids or their metabolites.

D. Maternal gonadal steroids in the egg can mediate maternal effects without interfering with the embryonic sexual differentiation processes. This is because sexual differentiation is mediated by gonadal hormones of the embryo whereas the maternal gonadal hormones are already substantially metabolized before the process of differentiation occurs (**chapter 4 and 5**). The conversion of active hormones to the less potent metabolites is thought to be irreversible, which could be experimentally verified using isotope labelled forms.

E. The embryo may not simply be a slave to the maternal signals but may play its own role in family conflict. This is because hormone conversion is dependent on laying order (**chapter 5)** and the embryo can modify its sensitivity to the hormone by adjusting its receptor levels **(chapter 6**). Whether the differences in hormone conversion based on egg laying order are a function of initial differences in hormone levels or due to differences in embryonic enzymatic activity is yet to be studied. This can be studied by adding hormones of interest to the first eggs to raise their levels to that of later laid eggs and then comparing their metabolism upon incubation.

F. The findings solve the problem of embryonic uptake of lipophilic hormones from the lipid rich yolk as (i) the hormones are partly converted to water soluble (conjugated) metabolites (**chapter 5**) and (ii) hormone receptors can be expressed at the interface between yolk and the embryo's circulation (**chapter 6**). Concerning the functions of steroid receptors in the extra-embryonic membranes, the following shall be studied further: (i) expression of steroid receptors in the extra-embryonic membranes at protein level, (ii) binding of yolk hormones to these receptors, (iii) subsequent cellular and physiological effects, (iv) in a control group blocking these receptors, but which depends on whether embryonic endogenous hormones also bind to these receptors and whether the blocker would enter embryonic body tissues, as these would interfere with functioning of embryonic endogenous hormones.

1.2 Eco-evolutionary significance of the mechanistic findings reported in this thesis

The active role of the embryo reported in this thesis may be significant for eco-evolutionary interpretations of hormone mediated maternal effects in following ways.

Since the time of discovery of systematic variation in maternal hormone deposition over the laying order and its correlation with variation in offspring behaviour (Schwabl 1993), it has been postulated that family conflict is likely to take place over function of maternal egg hormones (Groothuis et al. 2005b; Müller et al. 2007; Reed & Clark 2011; Winkler 1993) (also see (Del Giudice 2012; Godfray 1995; Trivers 1974; Wolf & Wade 2001)). Since these ideas have already been discussed extensively in the cited literature, here I would only highlight some key aspects of it.

As pointed by Müller et al (2007), the relationship between maternal hormones, offspring development and the environment is determined by (a) the relationship between the amount of maternal hormones deposited in the egg and the environment, which depends on maternal physiology and (b) the relationship between offspring development and the amount of maternal hormones in the egg, which depends on the offspring (embryonic) physiology and whether this is environmentally dependent. Variation in component (b) could be mediated already during embryonic development through processes such as up- or downregulation of hormone receptor density; conversion of a hormone to less or more potent metabolites; selective uptake of hormones, their metabolites, and other substances from the yolk; and increase or decrease of endogenous hormone production. The net outcome of the function of maternal egg hormones will thus depend not only on the costs and benefits for component (a) but also for component (b), and surprisingly, hardly any experimental information is available regarding the latter. The offspring is thought to benefit by differentially regulating its physiological processes in dealing with maternal hormonal signals under different environmental contexts in case the cost-benefit outcome is different between mother and offspring. Brood culling (Mock & Forbes 1994) and hatching asynchrony (Reed & Clark 2011) are classical examples where this might be the case, as explained below together with other contextual scenarios.

Under restricted resources, such as food, it might enhance the overall fitness of the mother to optimize the maximum brood size that she can raise, at the cost of one or more of the last hatched chicks that might be produced either because the food situation is difficult to establish at the time of egg production, or for insurance if one of the other eggs do not hatch. One possibility to achieve brood reduction is by hatching asynchrony. The eggs in a single clutch may hatch simultaneously or over an extended period of time, depending upon the onset of incubation by mothers. When incubation begins prior to laying of the later eggs of the clutch, it would lead to asynchronous hatching, which may lead to monopolisation of parental resources by the earlier hatched chicks. In species with hatching asynchrony, such as the rock pigeon and black headed gull, the mother might mitigate or reinforce the effects

of hatching asynchrony through variations in deposition of androgens, thereby maximizing her own fitness. In such a context, it might benefit the embryo that is the target for brood culling to mitigate the maternal effect and regulate the maternal hormonal signals for its own fitness if its exposure to maternal hormones would be unfavourable. This could be the case due to pleiotropic effects of hormones with potentially multiple costs and benefits for the offspring. Although the increased exposure to maternal hormones is mostly seen as advantageous for the offspring, it can confer costs too. For instance, an increased exposure of the embryo to maternal hormones could be beneficial for its growth and competitive abilities, but it could infer costs via suppressed immunity (Andersson et al. 2004; Groothuis et al. 2005a; Müller et al. 2005; Navara et al. 2005; Sandell et al. 2009) and/or oxidative damage (Galvan & Alonso-Alvarez 2010; Tobler & Sandell 2009; Tobler et al. 2013; Treidel et al. 2013). It has been argued that such cost-benefit outcomes of functions of maternal hormones are likely to be environmentally dependent (Hsu 2016, PhD thesis). For instance, a combination of parasite load and food abundance might be a more relevant cue, than either of them alone, for the offspring to allocate its own resources to growth or immunity, and hence it might optimize the use of maternal hormonal signals accordingly.

Another layer of complexity is added when the chick's inclusive fitness (i.e. the fitness of the entire current or future siblings, which will be genetically related to the focal chick) is considered, where the direct benefit of its own fitness might come at the cost of the fitness of its kin. At the same time, sibling conflict will arise through the relatedness asymmetries: individual offspring is more related to itself than to its siblings. Thus, the optima for effects of maternal egg hormones for the individual will not only depend on mother-offspring conflict, but also on the intricate balance between its individual fitness, inclusive fitness, and sibling competition. The extent of sibling rivalry will also depend on whether the costs and benefits of hormone mediated effects on any chick are felt by that chick alone or by the whole brood. For example, an increase in hormone mediated competitiveness (Groothuis & Schwabl 2002; Lipar et al. 1999) of an individual chick can be beneficial for the chick itself but can either divert food and other forms of parental care from the other chicks in the brood or increase the efforts of the parents in caring for the entire brood, which will carry costs to an individual offspring's inclusive fitness by reducing the number of surviving siblings produced from the current brood or from future broods. As another example, when an individual chick begs more (Smiseth et al. 2011), that chick may be fed more (an individual benefit), or the parents may bring more food but divide it between all offspring without reference to the begging behaviour of the focal chick. Similarly, the costs of begging may fall on the chick that is begging or on the entire brood, for example by attracting predators. Hence, in this latter example, the predator density or predation risk will be a relevant factor for overall effect of maternal hormone functions.

Additionally, because of possible sex differences in parental care, females may increase male parental investment by modifying begging behaviour, competitiveness, pre- and

postnatal growth of their offspring through the transfer of hormones to the egg (von Engelhardt & Groothuis 2011). Thus, the cost-benefit balance of function of maternal egg hormones may differ for components (a) and (b) (see above) contextually between species, such as species with or without hatching asynchrony, sex differences in parental care, altricial or precocial (precocial chicks being more independent than altricial from the time of hatching, thus potentially affecting the cost-benefit balance for the family), etc. Other examples of such contexts could be the quality of the father, sex of the offspring, parasite abundance, predation risk, etc. For instance, the mother might be depositing hormones differentially in relation to the quality of her mate and sex of the offspring (e.g. increased hormone deposition in sons of high-quality father), in which case there might be differences in the offspring's regulation of maternal hormonal signals based on its own sex and quality of its father.

To what extent maternal egg hormones and embryonic regulation of their effects actually play a role in such contexts is open for exploration. If the embryo were to play an important role in such contexts, an important question is how the embryo would perceive such contexts at such early stages of development. One speculation is that the information about such contexts might be perceived by the embryo in the form of egg composition itself other than hormones, such as nutrients (indicating food availability), antibodies (indicating parasite load), other hormones (indicating, for example, maternal stress level via stress hormone), and imprinting pattern of paternal alleles (indicating father's quality), etc. All these possibilities are open for experimental testing.

Finally, from the offspring's perspective, there might be substantial costs of the effect of maternal steroids if these affect the offspring's endocrine system, interfering with important processes such as sexual differentiation and/or reproductive performance later in life (Carere & Balthazart 2007). Our studies indicate that the embryo can potentially evolve mechanisms to protect itself from such effects when they are unfavourable.

1.3 Relevance of the findings in birds to other vertebrate taxa

In addition to the findings reported in this thesis on birds, a potential role of the embryo in regulation of maternal egg hormones has been extensively studied in reptiles (Bowden et al. 2002b,a; Paitz et al. 2012, 2017; Paitz & Bowden 2008, 2009, 2010, 2011, 2013, 2015; Paitz & Casto 2012; Paitz et al. 2011) and fish (Brown et al. 1988; Pri-Tal et al. 2011; Sopinka et al. 2017). The use of reptilian and avian species simplifies understanding the role of the embryo as, unlike in fish and mammals, there is no direct hormone transfer between the mother and the embryo after egg laying. It remains to examine further how the findings from the oviparous species would translate to mammalian species, including humans. For instance, it has been experimentally demonstrated in mammals that prenatal exposure to

excess glucocorticoids can affect the offspring physiology, leading to reduction in birth weight, increased likelihood of disorders of cardiovascular function, glucose homeostasis, activity of hypothalamic–pituitary–adrenal (HPA) axis and anxiety (Cottrell & Seckl 2009). The fetus can negate such effects to some extent by use of placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme (reviewed by (Del Giudice 2012)). In this regard, our findings of embryonic conversion of active hormones to less potent metabolites and downregulation of steroid receptors in the extra-embryonic membranes provide potential mechanisms by which the embryo can negate effects of maternal signals when they are unfavourable. If the metabolising enzymes are produced by the extra-embryonic membranes, this would suggest that functionally the extra-embryonic membranes in oviparous species may act similar to fetal placenta in mammals (also see (Cruze et al. 2012, 2013)).

Our findings of an early expression and regulation of steroid receptors in the EMs (**chapter 6**) have not yet been examined in mammalian placenta during very early developmental phases, and so is the effects of embryo's position in the litter (similar to laying order effect) in these processes. On the other hand, as in mammals both the embryo and the mother can affect each other throughout the fetal development (some examples of which were given on page 6), this might limit the comparative studies between birds and mammals. However, there might be a possibility that the avian embryo and the mother might be affecting each other behaviourally and physiologically not directly via transfer of hormones and/or their metabolising enzymes, but other traits such as vocalization and incubation behaviour (Dmitrieva & Gottlieb 1992, 1994; Gottlieb 1963, 1965, 1968; Gottlieb & Kuo 1965; Gottlieb & Vandenbergh 1968; Reed & Clark 2011).

CONCLUSION

In conclusion, I report the findings on so far neglected aspects of hormone mediated maternal effects in birds, both for maternal deposition and for embryonic modulation of egg gonadal steroids. Further, I discuss the results of these mechanistic studies for their potential implications in eco-evolutionary explanations of hormone mediated maternal effects. I hope that this work will stimulate further research for integrating proximate and ultimate approaches towards understanding the functions of maternal egg hormones, since mechanistic studies in this field have been lacking but are indispensable for making further progress and also for understanding the eco-evolutionary consequences of maternal egg hormone functions.