Explaining discrepancies in the study of maternal effects: the role of context and embryo

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The authors declare no conflict of interest.

Abstract

Prenatal maternal effects are increasingly recognized as important mediators in the development of individual differences during early sensitive or even critical periods. Hormone-mediated maternal effects in egg-laying species are a frequently used model to study such effects, mostly to test whether these increase maternal fitness. However, experimental evidence is inconsistent. This has led researchers to divert to other topics. In this review, we argue that from a Darwinian perspective one should however expect strong interactions between effects of maternal hormones with contextual cues, including environmental factors, embryonic modulation of maternal signals, offspring age and sex, and fathers' influence. Taking these into account may explain the inconsistencies and new experiments should reveal how the benefits and costs of maternal hormones and prenatal maternal effects in general play out in different contexts.

Introduction

The importance of non-genetic information transfer from parents to offspring and the response of the latter by developmental plasticity is increasingly recognized as an important factor for understanding both ontogenetic and evolutionary processes [1, 2]. It is now increasingly clear that the embryo is especially sensitive to signals from the mother at the early stage of development, and the actions of maternal signals can have a long-lasting organizing influence on its developmental trajectory [3]. However, such prenatal

maternal effects, and especially their underlying pathways, are often difficult to study as in most cases manipulation of the embryonic environment requires interference with the mother, inducing important confounding effects [3].

To avoid this issue, many researchers in the field of animal ecology study oviparous species in which the embryo develops outside the mother's body. This facilitates experimental manipulation on embryonic exposure to hormones without interfering with the mother. And as the maternal signals and resources are transferred into the egg before egg-laying, after which the embryo develops in a sealed environment, it is possible to measure prenatal exposure to these signals and calibrate treatment dosages accordingly. A lot of research in these species has focused on maternal steroid hormones that are deposited in the egg. From humans to insects, embryos are exposed to steroids of maternal origin, and the organizational effect of this exposure is well documented, for example by the work on sexual differentiation [4-6]. In particular, avian species, thanks to their well-known ecology and evolution and their large eggs with substantial concentrations of steroid hormones, have become well-established models to study hormone-mediated maternal effects from an eco-evolutionary perspective [3]. Indeed, systematic variation in steroid and non-steroid hormones in avian egg yolks have been demonstrated both within clutches over the laying sequence of eggs in the same breeding attempt [7-10], between nests of the same female [8-10], between females in relation to environmental factors [7, 9, 11, 12], and between species [13, 14]. This suggests that prenatal exposure to maternal hormones is not just an epiphenomenon but fulfills an important Darwinian function for the mother and, depending on parent-offspring conflict, the offspring as well. This is strengthened by evidence for independent regulation in the mother of hormone production for herself and the egg [15].

Maternal hormones and sensitive or critical periods

There is overwhelming evidence that injecting hormones in the avian egg before incubation affect a diversity of traits both prenatally and postnatally [7]. Interestingly, in the very first days of incubation, enzymes to metabolize maternal hormones and the relevant receptors are already present before the

53 embryo produces its own hormones [16-21, 76]. During this period, maternal hormones act as transcription
54 factors that can influence the expression of several genes, including their own receptors (e.g. androgens
55 [22, 76], glucocorticoids [23], and transmembrane transporters [24]). These actions alter offspring traits
56 after its hatching, long after the yolk and the hormones it contains have disappeared [e.g. 25], even well
57 into adulthood [e.g. 26]. Such effects of maternal hormones during the early embryonic development are
58 undoubtedly crucial, especially for some actions that are important during early development and canalize
59 further development (e.g. blood formation and neurulation [27]). For many traits, however, it is currently
60 unclear when the exact sensitive or critical period to the maternal hormones is and how this interacts with
61 the endogenous hormone production later in development. It is also unclear whether the maternal effects
62 are direct effects of the early hormone exposure, such as up- or down-regulation of the hormone receptors
63 later in life, or indirect effects (for example increased competitiveness may lead to faster early growth and
64 later dominance). Nevertheless, in the early stage of development the embryo may be more sensitive to
65 the maternal signals, for which it has receptors before the embryo starts its own endogenous production
66 (e.g. [17, 20, 76]). Because especially in early development, the developmental trajectories of subsequent
67 stages depend heavily on previous stages and on the particular stage in which the environmental signals
68 reach the embryo, any effect in the early stages will have unique and often multiple long-lasting effects
69 later in life. Therefore, although we cannot current pinpoint when, this stage may also reflect a critical
70 phase for these hormones to act.
71
72 *Discrepancies in previous studies*
73 Even within the field of hormone mediated maternal effects, many discrepancies from empirical
74 studies have arisen as to the patterns of hormone deposition in the egg and the effects of in ovo injection
75 of hormones [3, 7]. Part of the discrepancies could be due to experimental designs that manipulate
76 mothers rather than the hormones in the eggs, with the former methodology usually delivering smaller
77 effect sizes [30], partly due to the confounding effects from altered maternal physiology other than
78 hormone production. In addition, different studies use different dosages and maternal hormones could

have dose-dependent effects [31, 32]. More serious issues may arise when manipulation is induced outside the normal range for that species. That would lead to effects that are difficult to interpret, as evolution may not have prepared the subject for such deviations, leading to the study of effects and underlying pathways that would not occur in the wild. Although this may not be a problem for some medical research, it would not provide more general insight in principle and for the understanding of the causes and consequences of natural variation in maternal effects.

These discrepancies have raised doubts about the eco-evolutionary significance of hormone-mediated maternal effects and to some extent thwarted progress in this field. The aim of this paper is to stimulate further research in this field to increase our understanding of maternal effects and to provide a framework for such an approach. From an eco-evolutionary perspective, we argue that the possibility of context dependence as well as the role of the embryo itself have a great potential for further investigation and therefore focus on them in this review. Although we will mainly focus on hormone-mediated maternal effects, we believe this is a good model for providing insight in how to study maternal effects in general.

The relevance of context from an eco-evolutionary perspective

Hormones, like many other substances, are known to affect many different traits (pleiotropic effects). They circulate through the entire body as signals to help the organism to respond to changes in the environment or life-history phase, adjusting the phenotype by affecting morphology, physiology, perception and behavior in an integrative way [6]. As a consequence, trade-offs arise when beneficial effects, such as increased competitiveness or muscle strength, are accompanied by costs, such as increased metabolic rate or immune modulation [33, 34]. The cost-benefit balance may be strongly determined by environmental or non-environmental contexts. We broadly define “context” as both the environment external to the egg or chick, as well as the environment inside the egg in which the embryo is developing, and also other non-environmental contexts, such as offspring age and sex (Fig. 1). Although such contextual cues are rarely taken into account, they may vary hugely among different studies. This may likely lead to different outcomes among different studies.

105 Across species, the diverse life histories, ecological niches, and adaptations could also explain the
106 inconsistent effects of manipulated maternal hormones in different species (Fig. 1). Indeed, several studies
107 found evidence for the relationship between inter-specific variation in maternal yolk hormones and life
108 histories [13, 14, 35]. However, these are all correlational and we still lack direct experimental evidence
109 that maternal hormones exert different effects in species with different life histories.
110 Finally, there is often a conflict between parents themselves (i.e. father vs. mother), in which each of
111 the two would benefit from a higher investment in offspring by the other. As androgens stimulate begging
112 for parental food provisioning, it has been suggested that mothers may manipulate fathers' food
113 provisioning in monogamous species by depositing more androgens in her eggs [36-38]. Vice versa, fathers
114 can stimulate females' hormone deposition in eggs sired by him by his courtship behavior or secondary
115 sexual characters [39-40]. Recent evidence even suggests that fathers can affect offspring quality by the
116 hormones in his ejaculate, opening up a new exciting field on hormone-mediated paternal effects [41].
117
118 **Examples of contexts and suggested experimental designs**
119 From an evolutionary viewpoint, it is hypothesized that maternal hormones act as a maternal tool to
120 adjust the offspring to the prevailing or anticipated environment (hormones acting like a weather forecast
121 for the offspring [36, 42]). This makes sense, as the production of hormones in the mother is dependent on
122 the environment. For example, glucocorticoid production is increased upon exposure to stressors [43, 44],
123 gonadal hormone production depends on social stimulation [45], and thyroid hormone production varies
124 with temperature and food availability [11, 12]). Here, context comes in as a modulating factor in three
125 ways.
126 First, the external environmental context in which the offspring develops should match the
127 environment that was predicted by the maternal signal. This is often not actually tested but may be done
128 by, for example, manipulating stress or stress hormones in the mother and then rearing the offspring in
129 stress-free versus stressed conditions [e.g. 46, 47, reviewed in 48]. When the relationship between
130 maternal and offspring environment is not taken into account the negative effects observed in many

studies may in fact be caused by environmental mismatch [47, 48]. Second, whether maternal hormones provide an accurate "forecast" would depend on both the variation and predictability of the environment as well as how this scales to generation time [49, 50], which has hardly been taken into account. Third, if the mother really optimizes embryonic exposure to her signals and resources, any experimental manipulation away from this optimum would result in detrimental effects unless mothers were unable, due to environmental or intrinsic conditions (e.g. age or health), to optimize the signals and resources transferred to the offspring. In the latter case experimental manipulation of signals or resources may result in benefits for the offspring.

In all three cases above, the outcome of maternal hormones on offspring depends on whether the context encountered by the offspring matches the context anticipated by the mother, constituting a scenario of "anticipatory maternal effects" [42]. However, context-dependent maternal effects may be non-anticipatory, in which the effects only depend on offspring environmental context, regardless of whether it matches with mothers' anticipation. This may occur in extreme conditions, either when experimental manipulations of the maternal signal or the context fall outside of the species natural range of hormone concentrations, or when rare ecological events fall outside of what the species is adapted to. This would correspond to the "pathology scenario" (sensus [77]). It can also occur in natural contexts when a maternal signal has different effects on the offspring in different environmental contexts but overall lead to positive fitness return to the mother [51]. In this scenario, maternal hormone transfer may not depend on maternal environment, yet different outcomes on offspring may still come about due to the cost-benefit balance under different contexts [e.g. 12, 51]. For example, high metabolic rates only resulted in faster growth at a high food level but instead suppressed growth at low food level in brown trout (*Salmo trutta*, [60, 78]). Such food-dependent effects were also observed in rock pigeons (*Columba livia*) in which elevated yolk testosterone only benefited chick growth under good food conditions but resulted in higher mortality rate under poor food conditions [51]. Notice that this non-anticipatory context-dependent effect is not a type of carry-over effects of offspring's early-life environment (i.e. silver-spoon effects [77]), because offspring environment (e.g. food conditions) does not dictate the outcome of offspring phenotype.

157 This scenario could be more parsimonious to evolve because it takes mothers' ability to assess the current
158 context that may predict that of the offspring out of the equation. In fact, if such non-anticipatory context-
159 dependent parental effects are common, it could explain why an earlier meta-analysis only found weak
160 evidence for anticipatory parental effects [75]. In any case, manipulation of the prenatal environment
161 should be accompanied by manipulation of the postnatal environment.

162 In an egg, the embryo is provided with a variety of signals and resources such as hormones,
163 neurotransmitters, immune factors, vitamins, lipids and proteins [e.g. 10, 35, reviewed in 52]. These
164 substances constitute the direct environment in which the embryo is developing. The effect of one of these
165 may strongly depend on the other. For example, testosterone may increase early metabolic rate [53] but
166 metabolic rate is also affected by thyroid hormones [54]. In addition to the "internal environment" (i.e.
167 inside the egg) for the embryo, the external environment also influences the transfer of maternal
168 hormones. For instance, the level of social competition that the parents face may affect maternal
169 testosterone deposition [45] and ambient temperature thyroid hormone deposition [11]. Therefore, both
170 of these environmental factors and/or hormones should be taken into consideration when studying effects
171 on metabolic rate. Testosterone may also stimulate muscle growth [55, 56], but whether this can be
172 realized may depend on the resources in the egg or food provided by the parents post hatching, which in
173 turn depends on both parental quality and environmental food availability. Testosterone may modulate
174 immunity [57], but this effect depends for example on antibodies, vitamins and carotenoids provided by
175 the mother that in turn depends on various environmental and social factors [33, 58, 59]. As metabolic rate
176 and immune defense consume energy, both may have an effect on growth [34, 60] and thereby on the
177 competitive advantage of the offspring (Fig. 2).

178 Studying such interactive effects need a more complex experimental approach, in which more than
179 one environmental or egg factor is manipulated in a full factorial design. For example, an experiment of a
180 2×2 design should consist of a control group, a group in which factor A is elevated only (e.g. testosterone), a
181 group in which factor B is elevated only (e.g. food), and a group in which both A and B are manipulated.
182 One could also manipulate only one factor while taking advantage of circumstances in which B varies (such

183 as food availability in the field). The latter would be a good start but has the risk that the factor B (food
184 availability) is correlated with other factors such as parental quality or parasite load that in the end must be
185 controlled for.

186 To date, studies with such a factorial design are only few, but those that did do so seem to reveal
187 promising and interesting results. In grey partridges (*Perdix perdix*) chicks, Cucco et al. [61] found that
188 dietary carotenoid supplementation remedies the immunosuppression effects of a high dose prenatal
189 testosterone treatment. In Japanese quails (*Coturnix japonica*), elevation in yolk testosterone or
190 carotenoids alone both reduced hatching mass but increased blood reactive oxygen metabolites, while such
191 effects were offset when both testosterone and carotenoids were simultaneously elevated [62]. Making
192 use of the generally poorer food availability later in the breeding season, Muriel et al. [63] found that
193 elevated prenatal androgen exposure only reduced survival of spotless starling (*Sturnus unicolor*) nestlings
194 in the second broods. This is consistent with our data in the rock pigeons (*Columba livia*) that elevated yolk
195 testosterone increased nestling growth only in good food conditions whereas reduced nestling survival in
196 poor food conditions [51]. A few other experiments also found that maternal yolk androgens only benefited
197 chick growth when facing sibling competition or at competitive disadvantage [64, 65]. Such experiments
198 therefore should be able to reveal underlying mechanisms and explain discrepancies between different
199 studies. One might even expand such a factorial design to an n×n design [e.g. 66], to simultaneously
200 account for dose-dependent effects, but this may be only feasible with an insect model.

201 Albeit such studies are highly relevant, they do not fully address the question whether maternal
202 hormone deposition induce anticipatory maternal effects (see above). To this end, the factors A and B in
203 the above 2×2 design must represent the relevant factor in both the maternal and offspring environment
204 (i.e. a match-mismatch design) [50]. Such an approach has been adopted to study the interactive effects of
205 prenatal versus postnatal nutritional and social conditions [67-69]. Nevertheless, to our knowledge only
206 one unpublished study has addressed hormone-mediated maternal effects with such a design. In this study
207 Japanese quail hens were laying eggs under high or low temperatures (35 or 22 °C), inducing elevated
208 corticosterone production in the 35°C condition. After hatching of their eggs, half of the offspring of each

mother was raised in the high and the other half in the low temperature. It was found that at the age of 6 weeks the sensitivity of the corticosterone response to adrenocorticotropic hormone (ACTH) was clearly higher in the temperature-mismatched than in the matched situation (Rie Henriksen, PhD Thesis, University of Groningen, 2012). This suggests that the mismatch condition induced a higher stress sensitivity to temperatures other than what their mother may have programmed them for.

The role of the embryo itself

The eco-evolutionary approach mentioned above also points to an important role of the embryo itself. Parents may not necessarily have the same interest as their offspring, giving rise to parent-offspring conflict [70]. Because resources are often limited, individual offspring may want to extract as much resource from the parents as possible, whereas the parents may benefit from raising more offspring and therefore dividing their resources over all offspring at the cost of that individual offspring [70]. This would also lead to sibling competition and mothers may provide unequal resources or signals in favor of certain "core" offspring and induce mortality in marginal offspring [71, 72].

Traditionally, researchers usually assume that embryos accept all signals or resources transferred by mothers. However, under parent-offspring conflict, it is possible that offspring are not powerless, but have measures to counteract maternal signals to some extent [20-21, 73, 76]. This in itself may explain some of the discrepancies in the effects of maternal hormones in the literature and makes hormonal conversion by the offspring an intriguing non-environmental context. In avian egg yolks, the conversion of maternally derived hormones takes place already in the first week after fertilization [21, 79]. Interestingly, in pigeon eggs, this conversion differs between eggs that were laid by the same parents but in different position of laying sequence [20]. As in many bird species yolk hormones systematically vary across laying sequence [7, 36], the differential embryonic conversion of maternal hormones depending on the position of laying sequence opens an interesting possibility: If embryos are able to assess their position in the hatching sequence or other environmental factors such as temperature and food availability (e.g. cued by egg

234 composition, as it usually varies by laying order [35]), it suggests that the embryo is able to modulate the
235 maternal signal and plays its own role in the parent-offspring conflict already very early in development.
236 While maternal hormones are metabolized during the early stage of embryonic development [21,
237 74], the effects on offspring can be long lasting even into adulthood [26]. This thus begs the question
238 whether the effects of maternal hormones may differ over age, and exert sex-specific effects even though
239 the physiological variation of maternal hormones usually do not disrupt sexual differentiation [4, 5]. On the
240 one hand, sex-specific effects of maternal hormones are relatively well explored and a recent meta-analysis
241 suggested that maternal hormones exert a larger effect on males than females [30]). On the other hand,
242 whether effects of maternal hormones would diminish or even amplify over a lifetime is yet totally
243 unexplored.
244
245 **Conclusion**
246 Despite systematic differences in yolk hormone deposition in relation to environmental and social
247 conditions, experiments manipulating egg hormones have yielded inconsistent support, even within the
248 same species, for the idea that these hormone-mediated maternal effects are adaptive. This may be
249 explained by the fact that experimental designs were over-simplified, as from an adaptive perspective one
250 may expect a range of contextual cues that may modulate effects of experimental manipulations. Therefore
251 we need to develop hypotheses on how and which contextual cues would be involved and test with more
252 complex experimental designs. Also the question about whether and when there are sensitive or even
253 critical periods for the maternal signals to act on and to what extent these can be affected by these signals
254 themselves or other factors has not been addressed at all. In such experiments not only maternal
255 hormones should be manipulated, but also the relevant contextual cues. It also requires a better
256 understanding of when and how maternal hormones affect the phenotype, including underlying
257 physiological pathways and the role of the embryo, in order to delineate the relevant costs and benefits of
258 the pleiotropic effects of prenatal exposure to these hormones. This approach is relevant not only for
259 understanding hormone-mediated maternal effects in birds, but also for studies in other taxa and may be

generalized to maternal effects mediated by other substances such as carotenoids, immune modulating factors, and other crucial resources for offspring development. This approach is also likely to be relevant to other fields than behavioral ecology and evolutionary biology, and needs integration with for example endocrinology, developmental psychology and the new and rapidly upcoming field of evolutionary medicine, asking not only how but also why we become ill.

Acknowledgement

This work receives no funding. While drafting this manuscript, BYH is funded by the Ella and Georg Ehrnrooth Foundation, Finland.

Figure legends

Fig. 1. Scheme depicting how environmental (green boxes) and non-environmental (orange boxes) contexts may shape offspring phenotype (golden boxes) directly or indirectly so via maternal effects, such as maternally deposited yolk hormones (blue boxes) . Maternal environment may "forecast" offspring environment (accuracy depending on environmental predictability) and are "translated" into yolk hormone levels in the egg (or any other maternal substance) by the mother.

Fig. 2. Scheme depicting potential interactions between some major environmental factors (green boxes) and maternal-effect mediators (blue boxes) on offspring phenotype (golden boxes). Arrows represent the currently known relationship.

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