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Sources of variation in yolk hormone deposition: Consistency, inheritance and developmental effects

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ABSTRACT

Maternal effects occur when the phenotype of the mother affects the phenotype of their offspring. They are thought to have evolved to translate the environmental conditions experienced by the mother into adaptive phenotypic variation of the offspring. However, the integration of environmental cues allowing adaptive responses requires some form of plasticity that depends on the interaction of the maternal phenotype and her environment. In birds, maternal yolk hormones represent such a pathway for maternal effects, and their adaptive significance depends thus on the plasticity in maternal yolk hormone deposition.

We studied sources of variation in yolk testosterone deposition, focusing on the often neglected contribution of the (partly heritable) maternal phenotype. We investigated consistency and heritability of yolk testosterone deposition in captive canaries of which the F_1 generation was raised in foster nests and analyzed the potential effects of the early developmental conditions. We found significant female consistency across years in egg mass, yolk mass and total amount of yolk testosterone but not in yolk testosterone concentrations. Females varied the yolk testosterone concentrations of their eggs across years mainly via changes in yolk mass. The heritable variation in egg mass, yolk mass and amount of yolk testosterone but not yolk testosterone concentrations was within the range of previous studies, but not significantly different from zero. Finally, the growth of the daughters as nestling had a significant effect on their yolk testosterone deposition at adulthood indicating the transgenerational potential for environmental effects – via the effects of yolk hormones on offspring development.

1. Introduction

Maternal effects are defined as phenotypic variation in offspring that is a consequence of the mother's phenotype [33]. They are shaped by natural selection enabling adaptive responses of the offspring in heterogeneous environments, because of their potential to generate immediate phenotypic change via phenotypic plasticity [24,30]. The integration of cues from the maternal environment allowing adaptive responses requires plasticity of the maternal phenotype – that is shaped by the interaction of the maternal genotype and her environment. Thus, the evolutionary significance of maternal effects depends on (the genetic basis of) the maternal traits that generate phenotypic changes in offspring such as egg size in oviparous species (see [33] for a review). But maternal effects may have both a genetic and an environmental component, if the maternal effects are mediated by the influence of the maternal environment on the respective maternal traits [34]. Maternal effects also depend on the offspring responding to the maternal cues with developmental plasticity [26,33]. However, in the present work we will primarily be concerned with variation in maternal traits that generate maternal effects.

Maternal effects have been particularly well studied in oviparous species, where the embryo develops in the sealed environment of the egg, outside the mother's body. The egg provides essential resources for development representing an important environmental component to the development of a wide range of offspring traits. Both egg size and composition are largely determined by the mother. The egg represents, therefore, an important maternal trait that generates maternal effects. In birds, one particular egg component, maternally derived yolk hormones, have recently received increased attention in empirical work [6,14,15,39]. It is now widely acknowledged that yolk hormones represent an important pathway for maternal effects, given the numerous often persistent effects of yolk hormones on offspring phenotype that have been documented (reviewed in [6,15]. Yolk hormones – representing a means of a maternal effect – have been suggested to adjust the offspring to the post-hatching environment. A main focus of research has, therefore, been on identifying environmental sources of variation yolk hormone deposition (e.g. parasite exposure [43], food abundance [45], social environment [40], mate quality [8]; reviewed in [6,15]). However, as pointed out above, maternal effects have both

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a genetic and an environmental component, and their respective contributions strongly determine the short-term evolutionary significance of maternal effects. Thus, it is also crucial to improve our knowledge of the genetic basis of hormone-mediated maternal effects, because the potential to respond to selection is set by the degree to which a trait is determined by (parental) genes [5].

The first support for heritable variation in yolk hormone deposition, which is the trait that generates maternal effects, comes from a number of recent studies reporting intra-individual consistency in yolk hormone deposition [2,4,10,11,36,41,44]. Individual consistency is regarded as an upper-bound estimate for inheritance [20]. However, estimating individual consistency is difficult especially in the wild where females may return to their previous breeding habitat and/or mate with the same partner, which may yield higher estimates due to mate or territory resemblance. This possibly confounds previous results and it may require an experimental approach to tackle this problem.

However, the relative contribution of genes can ultimately only be inferred when comparing related individuals, but there is as yet little information available on heritable variation. Tschirren et al. [44] were the first to show significant heritable variation in yolk testosterone but not yolk androstenedione deposition in a wild bird population. Very recently it has now also been shown experimentally that this trait responds to (artificial) selection [29]. However, heritability typically shows large variation not only between traits but also between taxa, and through time (e.g. [22]). It has, therefore, to be shown whether and how similar levels of genetic inheritance occur in other species in order to improve our understanding of the evolution of hormone-mediated maternal effects.

Finally, the early developmental conditions that an individual experiences may have significant effects on the reproductive traits at adulthood [19], including egg size or clutch size in oviparous species (e.g. [17,38]). Interestingly, it has recently been shown that the early developmental conditions also impinge on yolk testosterone deposition at adulthood. Female zebra finches raised in experimentally enlarged broods deposited lower amounts of yolk testosterone [9]. Thus, the early developmental conditions may form an important but rarely considered source of variation in yolk hormone deposition.

Our study aims at improving our knowledge of the genetic basis of the maternal deposition of yolk testosterone, a maternal trait known to modulate offspring development in our study species {canary (*Serinus canaria*)} [28]. Knowledge of the heritable basis of yolk testosterone deposition is vital for our understanding of its evolutionary potential. Heritable variation will be estimated first based on intra-individual consistency in yolk testosterone deposition across years. By using a captive bird species, we reduce potential confounding effects of individual quality and environmental variation, as likely to be found in wild bird species, where high quality females may obtain higher quality mates and/or territories that may affect the estimation of consistency and heritability (e.g. [23]). Studying individual consistency allows us at the same time to identify potential sources of intra-individual variation. Second, we study the narrow sense heritability defined as the ratio of the additive genetic variance to the phenotypic variance [20] via mother–daughter resemblance in yolk testosterone deposition. Here, the offspring of the F_1 generation was raised in foster nests, in order to avoid mother–daughter resemblance through the experience of similar environmental conditions, which may lead to an overestimation of the heritability [22]. We also recorded the growth of the F_1 generation, which can vary substantially among broods and individuals despite ad libitum food conditions as found in captivity [37]. Quantifying the impact of the early developmental conditions on yolk testosterone deposition at adulthood is important, given the evidence from an earlier study [9] and its relevance for the evolution of hormone-mediated maternal effects. Finally, we included additional maternal traits (body mass, egg mass, yolk mass) in the analysis for comparison.

2. Material and methods

2.1. Breeding procedure

This study was conducted in 2007–2008 using two generations of *Fife Fancy* canaries. All animals were handled in accordance with good animal practice and experiments have been conducted according to Belgian legislation for animal experimentation (permit number 2006/19). In 2007, all birds that were assigned to the *parental generation* laid two clutches: the first clutch was initiated in March, 5 weeks after the light regime was changed to 14:10 L:D. This clutch was collected for hormone analysis. Mid May, all females were mated with a new partner, while housed under natural light–dark conditions. This second clutch was allowed to hatch in order to breed the F_1 generation. In 2008, all (live) individuals of the *parental generation* that sired a daughter in 2007, as well as the daughters of the F_1 generation (one daughter per female was randomly selected) were allowed to breed in March, 5 weeks after the light regime was changed to 14:10 L:D (breeding conditions). Females of the *parental generation* were mated with a male they had not been mated with before. In 2008, all clutches were collected for hormone analysis. All males used in this experiment were unrelated to their partner and only used once in order to randomize the potential effect of a – in our population as yet unidentified – male trait that may affect yolk hormone deposition.

In all breeding experiments, we kept the pairs in separate breeding cages equipped with nest boxes and nesting material. We provided the birds with canary seed mixture (van Camp, Belgium), water, shell grit, and cuttlefish bone ad libitum and twice weekly (after the chicks hatched daily) with egg food (van Camp, Belgium). Nests were checked daily and fresh-laid eggs were marked in order to keep track of laying date and laying position. Clutches were: either (a) collected for analysis. To this end we replaced the egg with a dummy egg on the day of laying. We weighed the eggs to the nearest 0.01 g and froze them for later hormone analysis. All dummy eggs were removed two days after clutch completion, the pair was split and the birds were returned to large flight cages, separated for sex. Or (b) incubated by their parents and allowed to hatch. At hatching, chicks were marked with a non-toxic pen for individual recognition, and a small blood sample (about 2–5 μl) was taken for molecular sex determination [13]. All female chicks were weighed (to the nearest 0.01 g) and cross-fostered, creating female-only broods of four similar weighed chicks. All male chicks were used in a different experiment. Thereafter, we measured the body mass (to the nearest 0.01 g) of all chicks early in the morning every day until day 20. By day 20 the growth curve has levelled off, and the chicks may fledge. Based on the body mass measures we estimated for each individual its asymptotic body mass (for details see Section 2.3). The asymptotic body mass represents an established, biologically interpretable measure of the individual growth trajectory and thus the quality of the early development ([32,27] in this species). We did not manipulate the early developmental conditions experimentally. However, individuals will likely differ in their growth despite ad libitum food conditions, which is thought to relate to variation in parental food provisioning and within-brood size asymmetries [37]. Relating the asymptotic mass to yolk hormone deposition will thus allow us to estimate the consequence of the early developmental conditions on yolk hormone deposition at adulthood.

We separated the chicks from their parents at independence (about 30 days old). All birds were subsequently kept in large single-sex aviaries.