Maternal Thyroid Hormones: An Unexplored Mechanism Underlying Maternal Effects in an Ecological Framework

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ABSTRACT

Maternal effects are currently acknowledged as important causes of transgenerational phenotypic variation and a potential mechanism to adapt offspring to predicted environments, thus having a pivotal role in ecology and evolution. Research in hormonal mechanism underlying maternal effects has focused heavily on steroid hormones. Other hormones, such as thyroid hormones (THs; thyroxine and triiodothyronine), have been largely ignored in ecological research until recently. We summarize the recent findings, identify knowledge gaps, and provide future research directions investigating the role of TH-mediated maternal effects in ecological context across taxa. Surprisingly, data on the sources of naturally occurring variation in maternal THs and their fitness effects are lacking in most vertebrate taxa. There is considerable variation in maternal TH levels in eggs across taxa. Avian egg THs show heritable variation, and data from fish and amphibians suggest female consistency in egg TH levels. In birds, variation in maternal THs was associated with important ecological factors, such as food availability and temperature. THs also showed intra-individual variation varying systematically within clutches. Importantly, exposure to maternal THs within naturally occurring range affected offspring fitness-related traits (growth and survival) in birds and fish. These findings make THs an interesting mechanism underlying maternal effects, which likely shape offspring phenotypes.

Keywords: thyroid hormones, maternal effects, development, endocrinology, T3, T4.

Introduction: Why and How Should We Study Maternal Thyroid Hormones in an Ecological Context?

Maternal effects are defined as the phenotype or environment of the mother affecting the phenotype of the offspring, and they are widespread among plants and animals (e.g., Mousseau and Fox 1998; Marshall and Uller 2007; Räsänen and Kruuk 2007; Badyaev and Uller 2009; Garland et al. 2017). Maternal effects are a crucial mechanism to generate phenotypic variation: potential mechanism to adapt offspring to predicted environments and postnatal maternal effects are known to have long-lasting effects on phenotype, behavior, and fitness (Maestripieri and Mateo 2009; von Engelhardt and Groothuis 2011). Depending on the fitness gains and costs for mother and offspring, maternal effects can be classified as adaptive (benefits to both mother and offspring), selfish (benefits to mother only), or transmissive (costs for both mother and offspring; i.e., maladaptive; sensu Marshall and Uller 2007). Recent meta-analyses revealed that adaptive maternal effects are likely to be rare (Uller et al. 2013).

The transfer of hormones from mothers to developing offspring has been identified as a key mediator underlying maternal effects across vertebrate taxa (mammals: Dantzer et al. 2013; fish: McCormick 1999; reptiles: Uller et al. 2007; birds: von Engelhardt and Groothuis 2011). For example, maternally derived testosterone in eggs of various reptiles, birds, and fish has inspired intensive research, yielding >600 publications during the past 20 yr. This line of research has focused on the causes and fitness consequences of naturally occurring variation in maternally derived steroid hormones (Groothuis et al. 2005; Groothuis and Schwabl 2008; von Engelhardt and Groothuis 2011; recently, e.g., Muriel et al. 2017).

However, steroids are not the only hormones that are transferred from mother to offspring. In this review we focus on the causes and consequences of variation in a key class of developmental hormones, the thyroid hormones (THs) of maternal origin (hereafter maternal THs). These include thyroxine (T4) and triiodothyronine (T3), which are important coordinators of development and metabolism (see below). In mammals, maternal transfer of THs across the placenta was confirmed in the 1980s (see, e.g., de Escobar et al. 1985 and references therein) and its clinical effects on children’s health and neurodevelopment are well established (e.g., Andersen et al. 2013). In the 1980s–1990s, maternal TH transfer to eggs was also described in fish (Kobuke et al. 1987; Brown et al. 1988; Mylonas et al. 1994) and birds (Prati et al. 1992; McNabb and Wilson 1997). In amphibians, there has been only one study that described egg TH levels (Xenopus laevis, African clawed frog; Morvan Dubois et al. 2006), and there are no published data from unincubated eggs of reptiles. Across ver...
the available data show substantial variation in the naturally occurring maternal TH levels among females (for examples of intraspecific variation across species, see table 1). There is likely also within-female variation, but this has rarely been explored. The sources of intra- and interfemale variation and the potential importance of maternal THs via eggs on offspring development and fitness have not been studied until recently, with the exception of fish (e.g., reviewed in Brown et al. 2014).

Such underexploration of variation in maternal THs is surprising considering the vast literature, for example, on maternal steroid hormones. The research that has been conducted on the maternal transfer of THs has primarily focused on clinical and biomedical context with humans and mouse models (Andersen et al. 2013; Medici et al. 2013; Korevaar et al. 2016), on domesticated species in the context of endocrine disruption (e.g., McNabb 2007; Van Herck et al. 2010, 2012, 2013), or on applied aquaculture/fisheries context (Brown et al. 2014). To date, an ecological/ecophysiological and evolutionary approach is largely lacking. Importantly, in many studies on the function of THs in early development, the differential contributions of maternal hormones and those produced by the organism itself have not been considered.

Our aim in this review is to emphasize the importance of THs of maternal origin as a mediator of maternal effects in an ecological context. Variation in maternal THs could lead to potentially adaptive, maladaptive, or permissive (i.e., interactions with other hormones) effects on offspring (see above). To shed light on the potential for any of these alternatives, we need to (1) understand how THs are transferred to offspring ("Mechanisms in Maternal Transfer of THs to Offspring"), (2) characterize both the environmental ("Ecological and Environmental Sources of Variation in Maternal THs and Associated Plasma THs") and genetic ("Genetic Variation in Maternal THs: Evidence") sources of the naturally occurring variation in maternal TH concentrations, as well as (3) understand the consequences of such natural variation on fitness-related traits in wild species and populations ("Fitness Consequences of Variation in Maternal THs"). We summarize the recent findings, identify knowledge gaps, and provide future research directions investigating the role of TH-mediated maternal effects in ecological and evolutionary context. We focus on egg-laying animals because, compared with mammals, their embryos are not affected by the continuously changing maternal environment, allowing studies of the independent effects of THs. Our goal is to highlight productive areas of research for future studies.

**Thyroid Hormones: An Overview**

The THs triiodothyronine (T3) and thyroxine (T4) and the hypothalamus-pituitary-thyroid (HPT) axis are conserved across taxa (Norris and Carr 2013). T3 is the biologically active hormone, whereas T4 acts as a prohormone. T4 and T3 (in smaller amounts) are produced in the thyroid gland, regulated via thyroid-stimulating hormone from pituitary. From the thyroid gland, T4 and T3 are released into circulation and transported (both bound to TH-binding globulins as well as unbound, i.e., free THs) to target tissues where they are taken up by TH membrane transporters. These include organic anion transporting polypeptides (OATPs), L-type amino acid transporters, and monocarboxylate transporters (MCTs). In target tissues, deiodinase enzymes further convert T4 to T3 and other TH metabolites, such as the inactive T3 (rT3). Type I deiodinase (Dio1) converts T4 to T3 and rT3, type II deiodinase (Dio2) converts T4 to T3, and type III deiodinase (Dio3) converts T3 to

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Thyroxine (T4)</th>
<th>Triiodothyronine (T3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Range</td>
</tr>
<tr>
<td>Human*</td>
<td>11.2 (1.5)</td>
<td>9.5–15.3</td>
</tr>
<tr>
<td>(Mus musculus)</td>
<td>11.6 (2.7)</td>
<td>8.4–17.1</td>
</tr>
<tr>
<td>Mouse</td>
<td>31.7 (3.8)</td>
<td>NA</td>
</tr>
<tr>
<td>(Ovis aries)</td>
<td>74 (15.6)</td>
<td>NA</td>
</tr>
<tr>
<td>African clawed frog</td>
<td>11.6 (7.7)</td>
<td>Threefold</td>
</tr>
<tr>
<td>(Xenopus laevis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coho salmon</td>
<td>16.3 (3.4)</td>
<td>NA</td>
</tr>
<tr>
<td>(Oncorhynchus kisutch)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zebrafish</td>
<td>2.96 (.83)</td>
<td>1.46–6.6</td>
</tr>
<tr>
<td>(Danio rerio)</td>
<td>3.06 (1.99)</td>
<td>1.77–6.14</td>
</tr>
<tr>
<td>Great tit</td>
<td>3.30 (1.27)</td>
<td>1.2–6.2</td>
</tr>
<tr>
<td>(Parus major)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rock pigeon</td>
<td>3.89 (1.56)</td>
<td>NA</td>
</tr>
<tr>
<td>Japanese quail</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Coturnix japonica)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Data on the range of variation in TH concentrations has not always been reported.

*First trimester (range [minimum–maximum] corresponds to the range in healthy females, according to international guidelines). In plasma, the absolute TH values vary across studies, depending on whether hormones bound to TH distributors or only unbound (free hormones) have been measured.

Day 16 during gestation.
T2. T3 binds to nuclear receptors (TRα and TRβ) that function as transcription factors. Recently, also nonreceptor-based pathways have been identified (Cheng et al. 2010). Details on the production, regulation, and metabolism of THs as well as their transporters and receptors have been reviewed elsewhere (e.g., McNabb and Wilson 1997; Power et al. 2001; Decuypere et al. 2005; McNabb 2007; Darras et al. 2015).

THs have key roles in development, metabolism, and reproduction. The earliest findings of developmental effects concerned the obligatory role of THs in amphibian metamorphosis (Gudernatsch 1912). THs produced by the embryo/fetus increase growth and are needed for differentiation/mutation of the central nervous system, muscle, skeletal, gut, and lung tissues in all vertebrates (McNabb and Wilson 1997; Decuypere et al. 2005; McNabb 2007; Darras et al. 2009; De Groef et al. 2013; Brown et al. 2014; Forhead and Fowden 2014). In adults, THs play a role in thermogenesis and metabolism of protein, glucose, and lipids in the initiation of gonadal development (e.g., McNabb 2007). They further contribute to life-history traits such as timing of egg laying and migration (e.g., Nishiwaki-Ohikawa and Yoshimura 2016; Perez et al. 2016). The timing of endogenous TH production varies across taxa. In precocial species (e.g., sheep, chicken) this happens earlier during development than in altricial species (e.g., mice, altricial birds such as passerines; McNabb 2007; De Groef et al. 2013). Importantly, before endogenous production, THs of maternal origin are present in eggs of oviparous species (see “Introduction”; table 1).

Mechanisms in Maternal Transfer of THs to Offspring

To understand plasticity in the levels of THs transferred from mothers to offspring via eggs and the potential for mothers to regulate hormonal exposure of their offspring (and thus offspring phenotype), one needs to know how THs are transferred from the mother and accumulate in the egg. Egg THs are derived from maternal circulation. Variation in egg THs may simply reflect variation in circulating TH levels or variation in clearance rate in female circulation, or alternatively, there may be some local regulation of yolk TH deposition. The latter option would facilitate adaptive egg TH regulation to produce a certain phenotype or to prevent transfer of damaging levels of THs. The link between circulating TH levels and egg TH levels has traditionally been studied by manipulating maternal plasma TH status via hormone implants/injections. Below we discuss evidence of the mechanisms in different taxa (summarized in table 2; see also fig. 1).

Mechanisms in Maternal Transfer of THs in Birds

Wilson and McNabb (1997) studied the link between female plasma and egg THs via experimental manipulation of circulating THs, using oral dosing of T4 (low or high concentrations vs. control) in Japanese quails (Coturnix japonica). Low TH treatment increased plasma T4 but not T3, whereas high TH treatment increased both plasma T4 and T3. In both low and high TH treatment, egg T4 and T3 levels increased compared with control, suggesting a direct link between plasma and egg TH. However, when individual plasma and egg T4 concentrations were studied within groups, a positive correlation between plasma and egg THs appeared in only the high TH group and not within the control group (Wilson and McNabb 1997). Second, when TH status in hen (Gallus gallus) was manipulated via an antithyroid drug (methimazole) to hypothyroid levels, both plasma T3 and T4 concentrations decreased. However, only egg T4, but not T3, decreased (Van Herck et al. 2013).

These studies suggest that a hyperthyroid maternal status may flood the system and lead to spurious positive correlations.

Table 2: Summary of the evidence, or lack of data, on sources of variation and effects of maternal thyroid hormones (THs) across oviparous vertebrates

<table>
<thead>
<tr>
<th>Evidence for</th>
<th>Bird</th>
<th>Fish</th>
<th>Reptile</th>
<th>Amphibian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfer of THs to eggs: correlations</td>
<td>+ /0^1,2</td>
<td>+ /0^1,4</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>between plasma vs. egg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Environmental sources of variation</td>
<td>Temperature^a</td>
<td>Location^a</td>
<td>Among female^a</td>
<td></td>
</tr>
<tr>
<td>in maternal THs</td>
<td>Food^a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within clutch^6,7</td>
<td>Stock variation^11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genetic sources of variation in maternal THs</td>
<td>Heritable T3^10</td>
<td></td>
<td>Female consistency^11,12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No heritable T4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effects of maternal THs on offspring</td>
<td>Neurodevelopment^13</td>
<td>Gene expression^19,20</td>
<td>?</td>
<td>Brain gene expression^12</td>
</tr>
<tr>
<td></td>
<td>Brain TH targets^4,5,15</td>
<td>Hatching + /0^16,17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hatching + /0^16,17</td>
<td>Growth + /0^16,18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Growth + /0^16,17</td>
<td>Early</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Metabolism + /0^16,17</td>
<td>Survival + /0^21,22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. Plus and minus signs indicate the sign of the correlation or effect, while a question mark indicates lacking data.

but in the naturally occurring range there might be potential for independent regulation between plasma and egg TH. The differential patterns in plasma T4 versus egg T4 and plasma T3 versus egg T3 further suggest that the two hormones might have independent regulatory mechanisms controlling transfer to eggs in avian taxa. However, the molecular mechanisms for such control are not understood.

Mechanisms in Maternal Transfer of THs in Fish

In fish, experimental manipulations of female plasma TH levels suggested a positive correlation between maternal plasma TH and egg TH levels (e.g., rockfish, *Sebastes schlegeli*: Kang and Chang 2004; rainbow trout, *Oncorhynchus mykiss*: Raine and Leatherland 2003). However, manipulating maternal TH resulted in hormone levels exceeding the natural range (up to 40 times), thus again making the interpretation of maternal egg hormone correlations in the natural range difficult (Brown et al. 1988; Ayson and Lam 1993; Raine and Leatherland 2003; Kang and Chang 2004).

Several studies have tried to elucidate the mechanism of TH transfer to eggs in fish but provided contrasting evidence. For example, THs have been found to be associated with vitellogenin transferred to eggs in some but not all studies (Tagawa and Hirano 1987; Flett and Leatherland 1989). Furthermore, Raine and Leatherland (2003) showed marked T3 efflux from the oocyte to the ovarian fluid if T3 concentration in oocyte was higher than in ovarian fluid. This study thus suggests trafficking of T3 in and out of the oocyte on the basis of concentration gradients across the oocyte cell membrane.

Mechanisms in Maternal Transfer of THs in Reptiles and Amphibians

We are unaware of any studies in reptiles or amphibians linking plasma TH and egg TH levels or studying the molecular mechanism underlying TH transfer to eggs.

Mechanisms in Maternal Transfer of THs to Offspring: Summary and Future Questions

To date, the potential for local regulation of TH transfer to eggs at the follicle level has received little attention in TH literature. From birds, the recent correlative studies suggest some potential for independent regulation, but in fish the available data are equivocal. If local regulation of egg TH levels occurs, potential candidates underlying the regulation of TH levels may include (see fig. 1) (1) variation in the expression of carrier molecules that bind THs and transport them into the egg (very low density lipoprotein, apolipoprotein D, transthyretin, and even vitellogenin) and receptor-mediated endocytosis, (2) variation in the expression of TH membrane transporters (MCT8, OATP1C1) in ovarian follicles, or (3) differential expression of deiodinases in the developing follicles (McNabb and Wilson 1997). Experimental approaches to further elucidate the underlying mechanisms could include radiolabeling studies, experimental manipulations of maternal circulating THs within the naturally occurring range during egg formation, and characterization of transporters and deiodinases in the developing follicles across taxa. Interestingly, for other maternal hormones, such as androgens, several studies suggest that androgen hormones are locally produced and regulated independently from plasma (Groothuis and Schwabl 2008).

Variation in Maternal THs: Why Is It Important?

Intraspecific variation in maternal TH levels arising from different sources could significantly affect offspring phenotype distributions, and thus it is important to understand the extent and the sources of such variation. Variation in egg TH levels could exist between or within females and across species. Such variation could reflect phenotypic plasticity (environmental origin) or genetic variation. Beyond recent studies in birds (see “Ecological Sources of Variation in Maternal THs in Birds”), variation in egg THs in the natural range in relation to environmental/maternal conditions remains largely unknown (summarized in table 2).
If TH levels in egg yolks can be decoupled from maternal plasma levels, then variable maternal TH transfer could function as a mechanism to program offspring phenotype (e.g., growth, metabolism) to match to the future environment (i.e., classical adaptive maternal effects). Alternatively, decoupling maternal plasma and egg THs could function as a buffer mechanism to prevent transfer of THs if they have potentially damaging effects. However, given that circulating THs are the only source of egg THs, some constraints are likely to arise. If there are such evolutionary constraints to independent regulation, we expect the variation in egg TH levels to be associated with similar environmental cues as in plasma THs. In such a scenario of (semi) passive transfer of THs from circulation, this variation could be maladaptive, permissive, or adaptive.

Ecological and Environmental Sources of Variation in Maternal THs and Associated Plasma THs

If egg hormone levels reflect circulating plasma levels, we predict that egg TH levels vary in response to the same environmental and ecological factors that cause changes in plasma THs. There are multiple key environmental and ecological factors that are associated with variation in plasma TH levels (see fig. 2). (1) Ambient temperature: THs are important in thermoregulation, and thus plasma THs vary in relation to ambient temperature (Cogburn and Freeman 1987; Comeau et al. 2000; McNabb 2007). (2) Food availability: plasma THs fluctuate with availability of food, potentially because they play a role in metabolism (Klandorf and Harvey 1985; Eales 1988; Reynolds et al. 2002). In wild populations, food availability locally may be affected by the level of inter- and intraspecific competition (and vice versa); thus, we hypothesize that density and competition could mediate unexpected effects via altered plasma and maternal TH status via affecting access to food. (3) Stress: the HPT axis is known to interact with the hypothalamic-pituitary-adrenal (HPA) axis; corticotropin-releasing hormone (CRH) acts as a common neuromodulator for both the HPT and the HPA axes, and regulation (inhibition or stimulation of HPT axis) depends on ontogenic stage and tissue type (e.g., reviewed in Kuhn et al. 1998; Castaneda-Cortes et al. 2014). Indeed, physiological stress has been found to affect plasma TH levels in wild animals (e.g., Angelier et al. 2016). In wild populations, multiple ecological stressors (such as competition, predation, and parasitism) increase plasma stress hormone levels (e.g., Baker et al. 2013). Such ecological stressors may thus influence plasma and maternal THs via HPA-HPT axis interactions. However, maternal stress is well known to also affect the transfer of glucocorticoids (GCs) to eggs (e.g., fish: Sopinka et al. 2017; birds: Henriksen et al. 2011; reptiles: Polich et al. 2018), and the vast literature reports a plethora of short- and long-term effects of prenatal exposure to stress hormones (e.g., Weinstock 2008; Schoech et al. 2011; Sheriff and Love 2013). Thus, it will be challenging to tease apart the direct effects of maternal THs on offspring versus indirect effects via variable transfer of maternal GC to eggs and TH-GC interactions during development. (4) Environmental iodine: iodine is needed for the biosynthesis of THs, and thus its availability affects plasma TH levels (McNabb et al. 1985; McNichols and McNabb 1987; Lewis 2004; Zhou et al. 2013; Hsu et al. 2016). We may speculate that in wild populations, variable iodine availability in diet across regions/habitats might thus affect plasma THs across or within populations. If such plasma TH variation directly translates to variation in maternal TH levels in eggs, it is likely to be nonadaptive or maladaptive. (5) Thyroid-disrupting chemicals: there is a wide range of chemicals that are known to disrupt the TH homeostasis mainly because of the high degree of structural resemblance to T4 (e.g., reviewed in McNabb 2007; Boas et al. 2012; Gutleb et al. 2016). They interfere with thyroid function at several levels by interacting with thyroid receptors, binding proteins, and hepatic clearance and subsequently affect circulating TH levels. These chemicals include polychlorinated biphenyls, dioxins, flame retardants (e.g., tetrabromobisphenol A, polybrominated diphenyl ethers, and polybrominated diphenyl ethers), various pesticides (e.g., dichlorodiphenyltrichloroethane hexachlorobenzene and nonylphenol), perfluorinated chemicals, phthalates, bisphenol A, and perchlorate. If variation in plasma THs induced by thyroid-disrupting chemicals is reflected in maternal yolk THs without any adjustment, this can be viewed as transgenerational transmissive effects (sensu Marshall and Uller 2007) of chemicals and certainly maladaptive. However, to our knowledge, the effects of TH-disrupting agents on maternal TH levels have not been explored in detail.

Ecological Sources of Variation in Maternal THs in Birds. In birds, recent studies provide evidence linking the variation in egg

Figure 2. Potential ecological and environmental variables that could cause variation in egg thyroid hormone (TH) concentrations, for example, via effects on maternal plasma THs. HPA, hypothalamic-pituitary-adrenal axis; HPT, hypothalamus-pituitary-thyroid; PCBs, polychlorinated biphenyls; PCDD, polychlorinated dibenzodioxins (dioxins); PBDEs, polybrominated diphenyl ethers; PBBs, polybrominated biphenyls; PFCs, perfluorinated chemicals.
TH levels and environmental and ecological factors. In wild great tits (*Parus major*) and captive rock pigeons (*Columba livia*), egg TH levels showed substantial variation among females, among clutches, and among eggs within clutches (Ruuskanen et al. 2016a, 2016d; Hsu et al. 2016). In a wild population of great tits, egg T4 was negatively associated with ambient temperature during egg formation (Ruuskanen et al. 2016d) and positively correlated with timing of breeding during the season (S. Ruuskanen and B.-Y. Hsu, unpublished data). However, when the effect of temperature was experimentally studied in captivity, no effect of ambient temperature before or during egg laying on egg THs was observed (Ruuskanen et al. 2016d), suggesting that other correlated factors (such as food availability) may have a causal effect.

The effect of food availability on egg THs was also experimentally studied in great tits and pigeons. Food restriction in rock pigeons significantly increased egg T3 concentration but reduced total T4 content compared with the control group (Hsu et al. 2016). On the contrary, a short-term food supplement (5 d prior laying the target egg) had no apparent effect on egg TH levels in great tits, although a substantial amount of supplementary food was consumed (Ruuskanen et al. 2016a). Thus, we may speculate that female and/or egg TH levels may not respond strongly to food supplementation but may respond more to food restriction (e.g., Harvey et al. 1981; Harvey and Klandorf 1983; Darras et al. 1995). Variation in egg THs in relation to resource-related ecological factors (such as density, intra- or interspecific competition) has not been studied. Furthermore, to our knowledge, the effects of ecological stress on maternal THs have not been investigated either.

A final factor that may cause variation in maternal TH is iodine availability. Limited iodine availability may alter thyroid function and cause variation in circulating THs. The potential limitation of iodine availability in diets of wild birds is not well understood. In domesticated birds, iodine availability may affect circulating TH levels, but no data on egg THs are available (e.g., McNabb et al. 1985; McNabb and McNabb 1987). However, given that the thyroid gland can store iodine for substantial periods, it is likely that only chronic iodine restriction will have large effects on TH metabolism.

In addition to among-female differences, intrafemale (intra-clutch) variation in maternal TH levels is also of great interest, because such patterns may explain phenotypic variation within families. Phenotypic differences among individuals from different positions of the laying order within a clutch have been reported (Lovern and Adams 2008; von Engelhardt and Groothuis 2011), and there could be multiple, mutually nonexclusive drivers for these differences. Other egg components, including steroid hormones, show distinct within-clutch patterns, with levels increasing or decreasing with laying order (Saino et al. 2002; von Engelhardt and Groothuis 2011). These patterns could result from trade-offs between resource allocation between self and eggs but may also reflect potentially adaptive functions to adjust the level of sibling competition. In great tits, egg T4 concentration increased with laying order, while no systematic pattern in T3 was observed (Ruuskanen et al. 2016a). In rock pigeons, T3 (but not T4) tended to increase with laying order (Hsu et al. 2016). It is currently not understood whether such patterns are caused by, for example, patterns in circulating THs during laying of the clutch, which are necessary for female reproductive functions (e.g., associated female preparation for incubation), or whether these patterns result from local regulation at each follicle. Importantly, the potential functional significance (if any) of the within-clutch variation is not understood and remains to be studied.

**Ecological Sources of Variation in Maternal THs in Fish.** Surprisingly, while egg THs have been repeatedly measured in fish (see “Introduction”), we are unaware of any studies characterizing the environmental or maternal sources of among-female or within-female variation in egg TH levels. Studies in fish report differences across species (Leatherland et al. 1989a; Tagawa et al. 1990; Power et al. 2001), stocks (rainbow trout: Leatherland et al. 1989b), or sites (bonnethead shark, *Sphyrna tiburo*: McComb et al. 2005). Interestingly, McComb et al. (2005) reported that the egg T3 and T4 concentrations in bonnethead sharks from Tampa Bay were consistently higher than from eggs from Florida Bay. The authors suggested that this may be due to higher temperatures in Tampa Bay and speculated that egg THs might thus explain the faster growth rates and metabolic rates at this site. However, no systematic investigation into the sources of variation in egg THs have been conducted.

**Ecological Sources of Variation in Maternal THs in Reptiles and Amphibians.** In amphibians, African clawed frogs were found to express threefold differences in egg TH concentrations among females (Morvan Dubois et al. 2006; Fini et al. 2012), but the ecological sources of such variation have not been studied. From reptiles, data on intra- or interspecific variation or its causes are lacking. Interestingly, in some reptile species, variation in nest temperatures could cause intraclutch variation in developmental speed, but hatching asynchrony is seldom reported. THs are suspected to play a role in metabolic compensation, which leads to synchronous hatching (McGlashan et al. 2017). Such studies never measured THs levels in unincubated eggs and did not try to separate the roles of maternally derived THs and endogenously produced THs before hatching.

**Genetic Variation in Maternal THs: Evidence**

In addition to environmental sources of variation (see “Ecological and Environmental Sources of Variation in Maternal THs and Associated Plasma THs”), quantifying the genetic variation in physiological and other phenotypic traits is important because it facilitates responses to selection and thus trait evolution.

**Genetic Variation in Maternal THs in Birds.** In Japanese quails, there were consistent differences among females in egg T4 concentrations (Wilson and McNabb 1997), although the data were not statistically analyzed. In great tits, heritability estimates were recently acquired using siblings of hand-reared, wild-caught individuals (Wilson et al. 2011), and there could be multiple, mutually nonexclusive drivers for these differences. Other egg components, including steroid hormones, show distinct within-clutch patterns, with levels increasing or decreasing with laying order (Saino et al. 2002; von Engelhardt and Groothuis 2011). These patterns could result from trade-offs between resource allocation between self and eggs but may also reflect potentially adaptive functions to adjust the level of sibling competition. In great tits, egg T4 concentration increased with laying order, while no systematic pattern in T3 was observed (Ruuskanen et al. 2016a). In rock pigeons, T3 (but not T4) tended to increase with laying order (Hsu et al. 2016). It is currently not understood whether such patterns are caused by, for example, patterns in circulating THs during laying of the clutch, which are necessary for female reproductive functions (e.g., associated female preparation for incubation), or whether these patterns result from local regulation at each follicle. Importantly, the potential functional significance (if any) of the within-clutch variation is not understood and remains to be studied.
edness (T3; $h^2 = 0.25$; Ruuskanen et al. 2016c). However, it is not understood whether heritability of egg TH levels simply reflects consistent and heritable variation in female plasma T3 levels (McLachlan et al. 2011) or whether it is independently mediated via heritable variation in deposition mechanisms or deiodinase enzymes converting T4 to T3 (see “Mechanism of Maternal Transfer of THs to Offspring: Summary and Future Questions”; fig. 1).

**Genetic Variation in Maternal THs in Other Vertebrate Taxa.** Morvan Dubois et al. (2006) reported that African clawed frog females showed consistency in egg TH levels. Leatherland et al. (1989b) showed variation among stocks of rainbow trout, which could relate to either environmental or genetic causes. We are unaware of any studies in fish, reptiles, or amphibians formally addressing or analyzing genetic variation in egg TH levels.

**Variation in Maternal THs: Summary and Future Directions**

We have shown that egg THs show substantial intraspecific variation both among and within females, which is associated with key environmental and ecological factors, such as food and temperature. However, the associations between other ecological variables (such as competition, predation, and parasitism), which could be linked to variation in THs via stress-thyroid interactions, have not been studied and may provide interesting avenues for further research. For example, stress hormones have been shown to suppress thyroid axis in adults (e.g., Kuhn et al. 1998; Angelier et al. 2016), leading to decreased circulating TH levels. In general, data from other taxa than birds are extremely scarce, and more studies on both the ecological and the genetic causes of the extensive variation (see tables 1, 2), especially from wild species and populations, are urgently needed.

**Fitness Consequences of Variation in Maternal THs**

Data from several lines of research provide evidence that maternal THs could play a substantial role on offspring development and phenotype in oviparous vertebrates. First, in humans and other mammals, exposure to maternal THs in early fetal stages is crucial for normal growth and development because clinical variation (variation outside normal range) during gestation leads to severe pathological consequences (e.g., reviewed in de Escobar et al. 2004; Zoeller et al. 2007; Patel et al. 2011). Given that many functions of THs are conserved across taxa, such effects are also likely to occur across oviparous taxa. Second, studies across vertebrates show that the early embryos express important molecular mechanisms of TH action (such as deiodinases, transporters, and receptors) before their endogenous TH production. Expression of these molecules responds to TH administration already during the first days of embryonic development (e.g., Geyaens et al. 2012; Morvan Dubois 2013; Darras et al. 2015; Van Herck et al. 2015; Too et al. 2017). This suggests that maternally derived THs could substantially affect early embryonic development and potentially have long-lasting effects on phenotypes and ultimately fitness.

Up to recently, the importance of naturally occurring variation of maternal THs on development and fitness has been poorly understood. In an ecological setting, it is important to understand whether the observed variation of maternal THs (1) within the naturally occurring range is (2) associated with variation in fitness-related traits in wild populations. In oviparous animals, there is substantial literature on the effects of exogenously manipulated THs on embryonic development, especially from fish. However, these studies often have major shortcomings hampering extrapolation of the role of maternal THs in an ecological context. In most biomedical and aquaculture studies, the doses are mostly pharmacological, and fitness attributes are rarely analyzed. Finally, many studies have manipulated TH levels days/weeks into embryonic development and thus do not explicitly test the effect of maternally derived hormones, which are present before embryonic development starts. The available data are summarized in table 2.

**Effects of Maternal THs on Birds**

In Japanese quails, experimental elevation of female circulating TH levels (above the naturally measured range) led to increased TH levels in eggs, accompanied with increased growth and differentiation of embryo pelvic cartilage (Wilson and McNabb 1997). However, such an approach may lead to other changes in the egg and thus direct manipulations of the egg TH levels are needed to confirm causality.

In chickens, direct in ovo manipulations of egg and embryo TH levels were conducted in the hyperthyroid range (greater than five- to 10-fold increase in egg hormone content) to study the effects on early brain development and regulation of TH availability in the brain (Darras et al. 2009). These studies suggested that THs affect the development of neural tubes (Flamant and Samarut 1998), and the expression of deiodinases and membrane transporters, but in a region- and age-specific way (Van Herck et al. 2012).

To our knowledge, the only studies that experimentally elevated egg THs (by injections into the yolk) within the naturally occurring range before incubation in species other than chicken and quail are two recent studies on great tits and rock pigeons (Ruuskanen et al. 2016b; Hsu et al. 2017). In these altricial species, individuals only start to produce THs after hatching (McNabb 2007), thus they rely on maternal THs during the whole embryonic period. In captive wild-type rock pigeons, elevated egg TH levels increased hatching success (Hsu et al. 2017), thus showing a clear fitness effect. However, in great tits from a wild population, no effect on hatching success was found (Ruuskanen et al. 2016b). In great tits, elevated egg TH levels showed a sex-specific effect on offspring growth (mass and skeletal size), increasing male and decreasing female growth (Ruuskanen et al. 2016b). In contrast, in rock pigeons THs reduced body mass, independent of sex, in the late nestling period (Hsu et al. 2017). Importantly, as body mass at time of leaving the nest is strongly associated with later survival in many bird species (e.g., Linden et al. 1992), such effects of maternal THs on body mass are likely to contribute to offspring (and thus maternal) fitness.
Furthermore, egg THs affected offspring physiology: elevated egg TH levels increased metabolic rates of female chicks at hatching compared with controls in rock pigeons (Hsu et al. 2017) but not in great tits (Ruuskanen et al. 2016b). Egg TH treatment also increased plasma T3 (but not T4) concentrations in female rock pigeons but reduced it in males (Hsu et al. 2017). The observed variation in metabolic rates (i.e., energy expenditure) may affect offspring fitness, especially in wild populations where resources are limited.

The observed inconsistency among the results from rock pigeons and great tits could be explained by species-specific sensitivity to maternal THs, for example, because of differential availability/distribution of deiodinases, TH transporters, or receptors across species. Importantly, embryos may not be passive recipients of maternal hormonal environment because final TH action is dependent on intracellular TH metabolism and selective TH uptake via transporters (e.g., McNabb 2007; Too et al. 2017). In precocial chickens, 8-d-old embryos responded to exogenous TH by altering expression of TH transporters and deiodinases: this means that they may compensate for low TH levels (e.g., more conversion of T4 to the active T3) or shield against high TH levels (e.g., more conversion of T4 to inactive rT3 and T2). However, 4-d-old embryos did not show changes in deiodinase (DIO2, 3) expression in brain (van Herck et al. 2012), which makes early embryos more vulnerable to changes in maternal TH supply. However, for altricial birds, the presence and timing of such molecular mechanisms are not known, and it could vary across species. Thus, characterization is urgently needed.

Alternatively, the inconsistent effects of THs across species could be explained by differential effects of egg THs, depending on the environmental conditions (context-dependent effects). To understand potential for any adaptive allocation of maternal THs, we need to study whether the effects of THs are dependent on those environmental conditions that explain variation in maternally derived hormone levels in the first place. For example, in rock pigeons, food restriction reduced egg T4 content (Hsu et al. 2016). Given that egg THs cause higher hatching success and altered metabolic rate in pigeons, we may speculate that the higher egg TH levels might be beneficial in only (expected) good conditions, in which offspring can bear such costs. However, without further data, such interpretations remain speculative.

Effects of Maternal THs on Fish

The effects of maternal THs on fish embryo and larval development have been studied largely in aquacultures (not in wild populations), using both immersion and direct injection to eggs as well as indirectly via manipulating maternal thyroid status. The study species include, for example, salmon (Oncorhynchus spp.), trout (Salmo spp.), striped bass (Morone saxatilis), tilapia (Oreochromis spp.), and zebra fish (Danio rerio). These studies show multiple effects of egg THs on embryonic and larval gene expression profiles, development, hatching, and growth rate. TH treatment also affected metamorphosis, pigmentation, and survival of the young (Brown et al. 1988; Ayson and Lam 1993; Power et al. 2001; Kang and Chang 2004; Raine et al. 2004; Walpita et al. 2007; Brown et al. 2014; Castillo et al. 2015; table 2). Recent studies using knockout of deiodinases (Bagci et al. 2015) and knockout of maternal TH uptake (Campinho et al. 2014) further showed altered gene expression and neural development. Similar to birds, both positive and negative effects of elevated maternal THs have been reported likely because of differences in dose, species specificity, and perhaps rearing environment. However, in the majority of the early studies, TH treatment was not applied to undeveloped eggs but only in later embryos/larvae and in high doses (five to 10 times above the naturally occurring range).

In contrast, Raine et al. (2004) studied the effects of direct T3 manipulation in undeveloped eggs in natural range on rainbow trout; T3 did not affect somatic growth, but mortality was higher in lower temperatures. TR receptor gene expression as well as ear structure was also affected (Coffin et al. 2012). Interestingly, some embryonic control over maternal THs was also observed (Raine et al. 2004). In a similar experiment, Walpita et al. (2007) found that in zebrafish, elevated maternal TH increased hatching success and pigmentation and affected TR receptor and Dio2 expression. One study reported enduring effects of egg THs: approximately 1-yr-old rainbow trout from experimental in ovo T3 elevation responded differentially to later increases in TH levels compared with controls (Raine et al. 2011). Such long-lasting effects can potentially influence fitness also in adulthood.

Effects of Maternal THs on Reptiles and Amphibians

The effects of THs of endogenous origin on development and metamorphosis in reptiles and amphibians are well known (e.g., Laudet 2011). However, to our knowledge, no studies manipulated egg THs within the naturally occurring range in the eggs before embryo development, mimicking maternal hormone availability in reptiles. In a few recent studies, THs were injected into eggs at later embryonic stages beyond naturally occurring levels. They showed that elevated THs can speed up prenatal development, increase heart rate (proxy of metabolic rate), and early posthatching growth in turtles (Sun et al. 2016; McGlashan et al. 2017). Furthermore, such treatments also decreased the expression of the androgen-converting enzyme aromatase and levels of estradiol and sex ratio (Sun et al. 2016), suggesting complex interactions among different groups of hormones.

In amphibians, to our knowledge, two experimental studies manipulated THs (elevated T3 and TH antagonist, NH-3) before the endogenous TH production in Xenopus laevis and Silurana tropicalis (Duarte-Guterman et al. 2010; Fini et al. 2012). Here, maternal THs modulated the expression of a number of TH target genes implicated in neural stem cell function or neural differentiation, genes in TH action (TR, Dio’s) and steroid metabolism. TH antagonist further diminished cell proliferation in the brain. However, external morphology or short-term mortality was not affected by T3 treatment (in 48 h; Duarte-Guterman et al. 2010). Experimental
studies manipulating THs after embryonic thyroid gland development also showed critical effects of the THs on, for example, brain development, such as brain morphology and increased tectal cell proliferation (Thompson and Cline 2016).

Effects of Maternal THs: Summary and Future Directions

This synthesis of the studies suggests that variation in maternally derived THs in naturally occurring range can affect offspring development, physiology, and fitness-related traits. Such fitness effects also imply that variation in maternal THs may be under selection. Surprisingly, data explicitly testing the effects of maternal THs on reptile and amphibian development and phenotype are almost lacking. Also, from fish most data originate from captive-bred or model species (e.g., laboratory stocks of zebra fish), limiting interpretation of the effects in ecological context. Studies from fish and birds suggest that there are both costs and benefits of high egg TH levels and that the direction of the effect may vary among species or sexes, with ontogeny and potentially depending on the context. Such effects may create trade-offs and contrasting selection pressures for the mother. However, because the embryo may not be a passive recipient of the maternal hormones, we need to characterize how and at what stage the embryonic conversion of THs may affect embryonic response to maternal THs.

Future studies should concentrate on the role of naturally occurring variation on offspring fitness-related traits in wild species and populations. We have limited knowledge on the potential long-lasting effects of maternal THs on adult survival and reproduction in wild species and populations. THs influence reproductive physiology, including timing of breeding (McNabb 2007; Dardente et al. 2014; Nishiwaki-Ohkawa and Yoshimura 2016), but the potential programming role of maternal THs on such traits is not well understood. Most importantly, to understand the context-dependent effects, experiments directly manipulating maternal TH levels should be combined with manipulations of the predicted environment, for example, food availability and thermal conditions. This would require full factorial experimental designs.

Conclusions

We are just at the beginning of understanding the significance of maternally derived THs within the naturally occurring range as an underlying mechanism for maternal effects in wild species and populations. In birds, fish, and amphibians, considerable interfemale variation in maternal TH levels in eggs was found. There was some indication of independent regulation of plasma and egg THs in birds but equivocal data from fish. The molecular mechanism of transfer of THs and potential local regulation of egg THs should be characterized. Variation in egg TH levels was explained by important ecological factors, such as food availability and temperature in birds. Furthermore, avian egg T3 showed heritable variation, and data from fish and amphibians suggested among-female variation in egg TH levels. However, sources of egg TH variation seemed to be largely unexplored beyond avian taxa: further studies are thus crucial to understanding the ecological role of maternal THs. Importantly, exposure to maternal THs within the naturally occurring range affected offspring fitness-related traits, such as growth, physiology, and survival in fish and birds, although data from wild populations and long-term fitness attributes are scarce. Surprisingly, data on the effects of maternal THs from reptiles and amphibians on fitness measures are almost completely lacking. These first findings make THs an interesting mechanism underlying maternal effects that may shape offspring phenotypes.

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