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Temperature-induced variation in yolk androgen and thyroid hormone levels in avian eggs

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ABSTRACT

Global warming has substantially changed the environment, but the mechanisms to cope with these changes in animals, including the role of maternal effects, are poorly understood. Maternal effects via hormones deposited in eggs, have important environment-dependent effects on offspring development and fitness: thus females are expected to adjust these hormones to the environment, such as the ambient temperature. Longer-term temperature variation could function as a cue, predicting chick rearing conditions to which yolk hormone levels are adjusted, while short-term temperature variation during egg formation may causally affect hormone transfer to eggs. We studied the effects of ambient temperature on yolk androgens (testosterone and androstenedione) and thyroid hormones (thyroxine and triiodothyronine) in great tits (*Parus major*) using data from unmanipulated clutches from a wild population and from aviary birds (*ad libitum* food) exposed to different experimental temperature treatments during five years. Both in the wild and in captivity, longer-term pre-laying ambient temperature was not associated with clutch mean yolk hormone levels, while the way androstenedione and thyroxine levels varied across the laying sequence did associate with pre-laying temperature in the wild. Yolk testosterone levels were positively correlated with short-term temperature (during yolk formation) changes within clutches in both wild and captivity. We also report, for the first time in a wild bird, that yolk thyroxine levels correlated with a key environmental factor: thyroxine levels were negatively correlated with ambient temperature during egg formation. Thus, yolk hormone levels, especially testosterone, seem to be causally affected by ambient temperature. These short-term effects might reflect physiological changes in females with changes in ambient temperature. The adaptive value of the variation with ambient temperatures pre-laying or during egg formation should be studied with hormone manipulations in different thermal environments.

1. Introduction

Global warming has induced substantial changes in the environment, but the mechanisms in different organisms to cope with these changes are not well understood. One potential, rarely studied, physiological mechanism is via maternal effects (Galloway, 2005; Visser, 2008; Meylan et al., 2012; Salinas and Munch, 2012; Shama et al., 2014). Maternal effects occur when the maternal phenotype/environment affects offspring phenotype, for example via different developmental signals or care for the offspring (Mousseau and Fox, 1998). Maternal effects generate phenotypic variation which could either facilitate or hamper adaptation to changing conditions (Räsänen and Kruuk, 2007; Uller, 2008; Marshall et al., 2008; Meylan et al., 2012). To truly assess whether adaptive allocation occurs we need to quantify variation in the mediators of maternal effects in relation to influential environmental factors.

Oviparous species, such as birds, are a good study system for investigating climate-driven variation in prenatal maternal effects as their eggs develop outside the mother's body, facilitating the measurement of maternal resources and signals. Steroid hormones

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in egg yolk, such as testosterone (T) and androstenedione (A4), are widely studied and vary substantially among clutches due to social and environmental factors (reviewed by Groothuis et al., 2005; Ruuskanen, 2015). They can affect both early and later offspring phenotype and survival (increasing growth, oxidative stress and metabolism; reducing immune response, Gil, 2008; von Engelhardt and Groothuis, 2011). However, other hormones, such as the thyroid hormones, thyroxine (T4) and triiodothyronine (T3), are present in egg yolk (McNabb and Wilson, 1997), but have rarely been studied in an ecological context. Nevertheless, maternal thyroid hormones (THs) are indispensable for normal development in other vertebrates (Power et al., 2001; Patel et al., 2011; Campinho et al., 2014) and have been shown to increase growth also in birds (Wilson and McNabb, 1997; Ruuskanen et al., 2016a).

For insectivorous passerine birds, such as our study species, the great tit (*Parus major*), temperature-related changes in the environment are important because their breeding success is heavily affected by timing of breeding in relation to the ambient temperature-dependent peak of insect food availability (Lack, 1950; Martin, 1987; Verhulst et al., 1995; Visser et al., 2006; Reed et al., 2013). Importantly, temperature patterns may be used as cues for timing of breeding (Visser et al., 2009, 2011; Schaper et al., 2012; Williams, 2012), making temperature a good candidate for explaining variation in maternal allocation. Furthermore, great tits are currently mismatched in egg laying in relation to the time of maximal prey availability; this is because the phenology of their prey advances faster in response to increased spring temperatures than the breeding time of great tits (e.g. Visser et al., 1998). However, the mechanisms (i.e. plasticity, microevolution) to keep up with this change are not fully understood and (hormone-mediated) maternal effects could play a role.

It is widely known that egg size, i.e. general resource allocation to eggs, decreases with ambient temperature in wild populations (Nager and Zandt, 1994; Christians, 2002; Lessells et al., 2002), but opposite, potentially adaptive patterns can arise where food is not limiting (Schaper and Visser, 2013). However associations between ambient temperature and egg composition, such as yolk hormones, have rarely been studied (but see Remes, 2011).

An ultimate, causal explanation of the variation in yolk hormones with ambient temperature is that *temperatures before egg-laying* may be used as *cues* for timing of breeding and/or the quality of the season (see above) and thus the conditions for offspring rearing: Yolk hormones are then adjusted to the different predicted conditions (predictive adaptive response; PAR, Nager et al., 1997; Gluckman et al., 2008; Groothuis and Schwabl, 2008). Indeed the effects of yolk hormones on e.g. growth may be dependent on environmental conditions (Boncoraglio et al., 2011; Kankova et al., 2014; Muriel et al., 2015). We hypothesize that as yolk androgen and thyroid hormones decrease developmental time and increase growth (McNabb and Wilson, 1997; Groothuis et al., 2005; in great tits Tschirren et al., 2005; Ruuskanen et al., 2016a) individuals starting to breed in warmer ambient temperatures, interpreted as cues for being closer to the food peak, should show higher clutch mean hormone levels to speed up the embryonic development of the chicks.

In addition to clutch mean hormone levels, such adaptive allocation may also concern variation in hormone levels across the laying sequence within a clutch. In great tits, androgen and thyroid hormone levels increase across the laying sequence but within-clutch patterns may also vary in relation to environmental conditions (Tschirren et al., 2004; Tobler et al., 2007; Groothuis et al., 2008; Heylen et al., 2012; Ruuskanen et al., 2016b). Such patterns in other species have been interpreted as a way to facilitate brood survival or brood reduction, in relation to hatching asynchrony (HA), or a bet-hedging strategy to increase offspring diversity (Schwabl et al., 1997; Laaksonen, 2004; Groothuis et al., 2005). Interestingly, HA increases in higher ambient temperatures and later clutches in tits (Verhulst et al., 1995; Vedder, 2012; Alvarez and Barba, 2014). Thus, we hypothesize that the increase (or decrease) of yolk hormones over the laying sequence may be more pronounced in warmer temperatures, either to provide more diversity in offspring phenotype or to account for greater HA.

Furthermore, there could be a causal effect of *short-term variation in ambient temperature during yolk formation* on yolk hormone levels, mediated through variation in circulating hormone levels in the female. For thyroid hormones (THs) this is likely, as plasma TH levels in birds is affected by ambient temperature (Kuhn and Nouwen, 1978; e.g. Cogburn and Freeman, 1987; reviewed by McNabb, 2007). Yolk THs originate from plasma and seem to correlate with circulating plasma thyroid levels (McNabb and Wilson, 1997; McNabb, 2007; with potential scope for independent regulation, Van Herck et al., 2013). For yolk androgens, there is inconsistent data for the correlation between circulating and yolk hormones, with some evidence for independent regulation (steroids being locally produced in the ovaries, e.g. Groothuis and Schwabl, 2008; Okuliarova et al., 2011).

Finally, the observed temperature effects could be mediated *indirectly* via food availability: insect food availability is strongly affected by ambient temperature, and temperature may further affect foraging behavior and food intake (e.g. Perrins, 1991; Winkler et al., 2013). However, studies with experimentally altered food supply shown contradictory effects on yolk hormone levels (e.g. Benowitz-Fredericks et al., 2013 and ref therein). Thus, to separate the effects of food and temperature, controlled experiments are needed.

We studied the effect of variation in ambient temperature on the levels of two influential yolk hormone classes, androgens and thyroid hormones in great tits. We used data from unmanipulated clutches of wild birds to describe patterns in relation to ambient temperature. However, patterns associated with temperature may be mediated via food availability; thus, to reveal direct causal effects of temperature on yolk hormone levels, we analyzed the eggs of captive birds with *ad libitum* food, exposed to different experimental temperature treatments. This temperature-controlled data from a wild species is a truly unique dataset. We analyzed the effect of *pre-laying ambient temperatures* on both clutch mean hormone concentration and patterns across the laying sequence. We further analyzed the effect of temperatures *during yolk formation*. As captive birds are not energetically limited, we predict weak association with ambient temperature in captivity if temperature-related variation in yolk hormone levels is mediated indirectly via food availability.

2. Methods

2.1. Study species

The great tit is a model species in ecological and evolutionary research. Importantly, maternally derived androgen and thyroid hormones in eggs have been found to affect offspring growth and dispersal (Tschirren et al., 2005; Ruuskanen et al. 2016a; Tschirren et al., 2007; Tschirren and Richner, 2008; Podlas et al., 2013), and vary with multiple environmental factors (Tschirren et al., 2004; Groothuis et al., 2008; Remes, 2011; Ruuskanen et al., 2016b).

2.2. Data from a wild population

The correlative field data was collected in 2013 from a population with 125 nest-boxes in Bennekom, the Netherlands. Nest-building was monitored from end of March twice a week.