General and Comparative Endocrinology 232 (2016) 145–150

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Maternal corticosterone deposition in avian yolk: Influence of laying order and group size in a joint-nesting, cooperatively breeding species ☆

Gregory Schmaltz [a,b,*], James S. Quinn [a], Stephan J. Schoech [c]

[a] McMaster University, Department of Biology, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada
[b] University of the Fraser Valley, Department of Biology, 33844 King Road, Abbotsford, BC V2S 7M8, Canada
[c] University of Memphis, Department of Biological Sciences, 3774 Walker Avenue, Memphis, TN 38152-3560, USA

ARTICLE INFO

Article history:
Received 31 October 2015
Revised 4 March 2016
Accepted 22 April 2016
Available online 23 April 2016

Keywords:
Corticosterone
Yolk steroid hormone deposition
Smooth-billed ani
Communal breeding
Social stress
Radioimmunoassay

ABSTRACT

Glucocorticoid hormones play a key role in day-to-day adjustments to fluctuating metabolic needs. These hormones also mediate physiological and behavioral responses to stressful events, allowing individuals to cope with stressors. Various environmental insults, such as a food shortages, predation attempts, and agonistic encounters often elevate plasma glucocorticoid levels in vertebrates. Because exposure to maternally-derived (via circulation or egg) glucocorticoids may be detrimental to the developing embryo, maternal stress can have negative carryover effects on offspring fitness. We examined corticosterone, the primary avian glucocorticoid, concentrations in egg yolk in a plural-breeding, joint-nesting species, the smooth-billed ani (*Crotophaga ani*), in which females compete among themselves to lay eggs in the final incubated clutch. We investigated whether yolk corticosterone levels varied with laying order and group size. Because egg-laying competition leads to physiological and social stress that is intensified with group size and laying order, we predicted that yolk corticosterone levels should increase from the early to the late egg-laying period and from single female to multi-female groups. In this two-year field study, we found that yolk corticosterone levels of late-laid eggs within the communal clutch were higher in multi-female groups than in single female groups. Results from this study suggest that laying females experience higher levels of stress in multi-female groups and that this maternal stress influences yolk corticosterone concentrations. This study identifies a novel cost of group-living in plural-breeding cooperatively breeding birds, namely an increase in yolk corticosterone levels with group size that may result in detrimental effects on offspring development.

1. Introduction

Avian eggs contain maternally-derived hormones that can have significant effects on embryonic development, post-hatching growth, and offspring survival (for reviews see Groothuis et al., 2005; Groothuis and Schwabl, 2008; Williams and Groothuis, 2015). The allocation of hormones to the egg may vary in response to a number of factors including laying order, paternal quality, and food availability (Gil, 2003; Groothuis et al., 2005; von Engelhardt and Groothuis, 2011). For instance, yolk testosterone levels vary with laying order and can affect nestling begging behavior, growth, and social status or interactions among nest-mates (Gil, 2003; Groothuis et al., 2005; Schwabl, 1993, 1996). Some researchers interpret these studies to hypothesize that females use yolk hormone deposition to adjust adaptively their reproductive investment in response to the current conditions (Schwabl et al., 1997; Stoleson and Beissinger, 1995; see von Engelhardt and Groothuis, 2011; and Williams and Groothuis, 2015 for reviews). While findings from research in this area are exciting, caution and further studies are needed before one can assume causal links between yolk hormone levels and a variable of interest, given contradictory findings across studies and the relatively few published works from which to draw meaningful conclusions.

In his seminal paper, Schwabl (1993) reported that egg yolks contain measurable amounts of sex steroid hormones, such as androstenedione, testosterone, 5α-dihydrotestosterone, and 17β-estradiol, as well as low levels of the adrenal steroid hormone, corticosterone (CORT). Because relatively low levels of CORT are present in eggs, this steroid hormone has received much less attention than androgens (Groothuis et al., 2005), with a small number of published studies that have focused on yolk CORT levels (Larsen

☆ The methods of this study are based on a study first reported in Schmaltz et al. (2008b).

* Corresponding author at: University of the Fraser Valley, Department of Biology, 33844 King Road, Abbotsford, BC V2S 7M8, Canada.
E-mail address: gregory.schmaltz@ufv.ca (G. Schmaltz).

http://dx.doi.org/10.1016/j.ygcen.2016.04.022
0016-6480/

et al., 2015; Love et al., 2005, 2008; Saino et al., 2005; Vallarino et al., 2012). Furthermore, our understanding of yolk CORT effects may be further limited by the methodology that has been commonly used to measure CORT. For instance, Rettenbacher et al. (2009) suggest that yolk CORT levels may have been overestimated in various studies, as much of the CORT measured in eggs may actually be progestens, as they exhibit considerable cross-reactivity with the antibodies that have commonly been used. Given that progesterone is a common early product in the production pathway of all steroids, it and related progestens can be expected to be abundant in yolk (Lipar et al., 1999; Paitz and Bowden, 2010; Rettenbacher et al., 2009).

Knowledge of how CORT is incorporated into the egg yolk and whether or how it subsequently affects development is limited, despite the fact that corticosterone is an important hormone that can influence both maternal and offspring phenotype (Groothuis and Schwabl, 2008; Poisbleau et al., 2009; Rettenbacher et al., 2005; Schoech et al., 2011; Williams and Groothuis, 2015). Pre- or peri-natal exposure to elevated levels of maternal or endogenous glucocorticoids can have negative effects, both short-term effects upon embryonic development (Eriksen et al., 2003; Heiblum et al., 2001; Love et al., 2008; Mashaly, 1991), and long-term effects upon other traits, such as offspring body mass (Love et al., 2005, 2008; Saino et al., 2005), growth (Hayward and Wingfield, 2004; Heiblum et al., 2001; Love et al., 2005), sensitivity to stress (Hayward and Wingfield, 2004; Schoech et al., 2011; Welberg and Seckl, 2001), and behavior (Lesage et al., 2004; Rubolini et al., 2005; Schoech et al., 2009). Although the zona reticularis of the adrenal cortex is a known source of androgen production, no evidence of ovarian CORT production has been reported (Groothuis and Schwabl, 2008). Thus, any CORT within the yolk almost certainly is of adrenal origin that has moved to the ovary, and hence the yolk, via the maternal circulatory system. Therefore, yolk CORT levels should, to some degree, reflect maternal plasma levels of CORT given that it is a lipid-soluble hormone that likely enters the yolk by passive diffusion (Almasi et al., 2012; Groothuis et al., 2005; Hayward and Wingfield, 2004; but see Rettenbacher et al., 2005). However, the lipid-rich composition of yolk combined with the highly vascularized nature of ovarian follicles and the lipophilic nature of steroid molecules establishes a scenario where egg yolk could in effect serve as a CORT 'sink'. Because the affinity of CORT for yolk is much greater than it is for aqueous plasma, CORT might have a propensity to move into the yolk, thus concentrating therein (Groothuis and Schwabl, 2008). Yolk CORT levels almost certainly represent accumulation over the several day period of yolk deposition following recruitment of a given follicle. As a result, yolk CORT may represent a time-integrated measure of maternal CORT (Cook, 2012). Therefore, yolk CORT levels needn't necessarily reflect a laying female's current state, rather, levels within the egg may reflect the environmental conditions that the female experienced during yolk formation (Love et al., 2005; Saino et al., 2005). For example, female barn swallows (*Hirundo rustica*) that were exposed to a predator during egg-laying produced eggs with higher concentrations of CORT than controls (Saino et al., 2005). Further, embryos that were exposed to higher CORT levels were less likely to successfully hatch, and of those that hatched and subsequently fledged, the young were smaller than controls (Saino et al., 2005). Therefore, stressful conditions experienced by mothers may influence egg quality and offspring phenotype, which in turn, may negatively affect maternal fitness (Rubolini et al., 2005; Saino et al., 2005; Sinervo, 1998).

In this study, we examined yolk corticosterone concentrations in the smooth-billed ani (*Crotophaga ani*), a plural-breeding, joint-nesting communally breeding species. Anis are characterized by an unusual breeding system wherein groups of between one and seven socially monogamous pairs share a single nest (Quinn and Startek-Foote, 2000). Intense egg-laying competition for access to the final incubated clutch of eggs occurs in multi-female groups with eggs laid early having high probabilities of loss because of egg tossing and burial beneath nest materials and subsequently laid eggs (Quinn and Startek-Foote, 2000; Schmaltz et al., 2008a). This egg-laying competition intensifies as group size increases and both the number of eggs laid and lost per female increase with group size (Schmaltz et al., 2008a). We investigated whether yolk CORT levels varied with group size and laying order (i.e., laying order for all eggs within a specific communal clutch). We predicted an increase in yolk CORT levels from the early to the late laid eggs because costs associated with egg production and the ensuing physiological stress likely increase during egg production (Salvante and Williams, 2003; Williams, 2005). Such an increase in yolk CORT levels with laying order has been reported recently in lesser black-backed gulls (*Larus fuscus*; Larsen et al., 2015). Females in single-female ani groups do not experience the potentially stressful agonistic within-group interactions than their multi-female counterparts experience. Further, females in single female groups lay fewer eggs than their multi-female counterparts. Therefore, we also predicted that when compared to multi-female groups, yolk CORT levels in single-female groups should (1) be generally lower and (2) not exhibit marked laying order increases.

2. Material and methods

2.1. Study sites

We studied smooth-billed anis at the Cabo Rojo (17°59′N, 67°10′W, elevation from 2 to 42 m) and Laguna Cartagena (18°01′N, 67°06′W, elevation from 55 to 71 m) National Wildlife refuges in south-western Puerto Rico during the rainy season when most ani breeding occurs (September-January, 2003-2004 and 2004-2005, hereafter denoted as the 2003 and 2004 seasons, respectively). The south-western portion of Puerto Rico has a dry tropical climate and both Cabo Rojo (587 acres) and Laguna Cartagena (794 acres) refuges contain second-growth dry scrubland, open, and disturbed habitats that attract breeding anis (Loflin, 1983; Quinn and Startek-Foote, 2000).

2.2. Field procedures

Details about group size determination and nest check regime are provided elsewhere (Schmaltz et al., 2008a). In brief, we checked every active nest daily during the egg-laying period and marked every egg with non-toxic markers to indicate egg number in the laying sequence of a given nest. We sampled eggs that were among the first or last 20% of a group's clutch (i.e., early- *versus* late-laid eggs) and did the same in nests with single female breeders. Therefore, from this point onwards, we use the terminology 'group egg-laying order' instead of 'egg-laying order'. We collected yolk samples from freshly laid, yet to be incubated eggs by inserting a 25 gauge needle through the eggshell at the middle of the long axis of the egg, following the method pioneered by Schwabl (1993). We inserted the needle halfway within the egg to reach the center of the yolk (see Lipar et al., 1999) and extracted a small sample (approximately 40 mg) of yolk with a 1 ml disposable syringe. We sealed the insertion hole with New-Skin® liquid bandage (Medtech, Jackson, WY, USA) and returned the egg to its nest. We transferred yolk samples to 1.5-ml Eppendorf® tubes that were kept cool on ice until return to the lab where they were frozen and stored until shipment overnight on ice to the University of Memphis for assay (see below). To verify the uniformity of the sampling method, we also collected samples by yolk biopsies from 14 freshly laid intact eggs that had been recently 'tossed' from nests not under study (0–2 day-old eggs). We froze these 14 eggs,