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Red-winged blackbirds (*Agelaius phoeniceus*) with higher baseline glucocorticoids also invest less in incubation and clutch mass

Laura A. Schoenle [a,*], Alana M. Dudek [a], Ignacio T. Moore [a], Frances Bonier [a,b]

[a] *Department of Biological Sciences, Virginia Tech, 1405 Perry St., Blacksburg, VA 24061, United States*
[b] *Biology Department, Queen's University, Biosciences Complex 3523, Kingston, ON, K7L 3N6, Canada*

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ABSTRACT

Glucocorticoid hormones facilitate responses to environmental challenges by mediating diverse physiological and behavioral changes, including resource mobilization and altered reproductive effort. Elevated glucocorticoids might indicate that an individual is facing high levels of environmental challenges and thus, elevated concentrations might be associated with reduced fitness (CORT-fitness hypothesis). Alternatively, the energetic demands of reproduction might be a challenge that requires elevated glucocorticoids to mobilize resources to support reproductive effort, ultimately increasing reproductive investment and fitness (CORT-adaptation hypothesis). Investigations of glucocorticoid-fitness relationships have yielded mixed results. Variation in the direction of this relationship could be caused in part by differences in the contexts in which the relationship was assessed. Incorporating context, such as life history stage, could be key to understanding the role of glucocorticoids in influencing fitness outcomes. We investigated the relationship between corticosterone and reproductive effort and success within a single life history stage: incubation of eggs. In an observational study, we measured baseline corticosterone in incubating female red-winged blackbirds (*Agelaius phoeniceus*), monitored incubation behavior, and determined hatching success for each nest. Incubating birds with higher baseline corticosterone concentrations had more frequent, shorter incubation bouts and spent less time overall incubating their clutches of eggs than birds with lower corticosterone concentrations. Elevated corticosterone was also associated with lower clutch mass, but neither corticosterone nor incubation effort were correlated with hatching success. Although experimental tests are needed to establish causation, these results suggest that during the incubation period, corticosterone might shift resource investment towards self-maintenance, and away from current reproductive effort.

1. Introduction

When an individual is faced with immediate or chronic environmental challenges, glucocorticoid hormones can mediate a suite of physiological and behavioral responses, including shifts in resource acquisition and mobilization, and reproductive behaviors (Romero, 2004; Sapolsky et al., 2000). The role of glucocorticoids in facilitating the response to environmental and social challenges has lead to interest in the relationship between these hormones and fitness (e.g., Breuner et al. 2008, John-Alder et al. 2009, Ebensperger et al. 2011, Vitousek et al. 2014, Madliger and Love 2016). Initially, elevated baseline glucocorticoid concentrations (i.e., levels not influenced by an immediate threat/acute stressor) were thought to signal that an individual was in poor condition or facing challenges (Arlettaz et al., 2007; Bonier et al., 2009a; Wikelski and Cooke, 2006). The CORT-fitness hypothesis is built on this assumption and asserts that glucocorticoids will negatively correlate with fitness and reproductive effort because individuals facing greater environmental challenges will have higher concentrations of circulating glucocorticoids (Bonier et al., 2009b). However, the relationship between baseline glucocorticoids and survival and/or reproduction appears to be highly variable across contexts (Bonier et al., 2009a; Crespi et al., 2013). Even within a single species, the direction of the relationship between glucocorticoids and fitness can vary across life history stages (Bonier et al., 2009b; Ouyang et al., 2013, 2011), environmental conditions (Riechert et al., 2014; Schultner et al., 2013), or among individuals with different reproductive strategies (Lancaster et al., 2008). For example, in house sparrows (*Passer domesticus*), both males and females with low glucocorticoid concentrations prior to breeding and high glucocorticoid concentrations during breeding produced the most offspring (Ouyang et al., 2011).

To account for the variation in the direction of glucocorticoid-fitness relationships, Bonier et al. (2009a) proposed an extension of the CORT-fitness hypothesis: the CORT-adaptation hypothesis. The CORT-adaptation hypothesis suggests that if reproduction is energetically challenging, then glucocorticoids could mediate physiological and behavioral changes in support of meeting that challenge, resulting in increased

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[*] Corresponding author.
E-mail addresses: laura.schoenle@gmail.com (L.A. Schoenle), alana33@vt.edu (A.M. Dudek), itmoore@vt.edu (I.T. Moore), bonierf@queensu.ca (F. Bonier).

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reproductive effort and success (Bonier et al. 2009a). In support of this hypothesis, there is a growing body of evidence that glucocorticoids can be associated with increased reproductive success and thereby, fitness (e.g., Bonier et al. 2009a, Escribano-Avila et al. 2013, Riechert et al. 2014). We might expect to find support for the CORT-adaptation hypothesis when reproductive effort is the primary factor influencing individual variation in glucocorticoid concentrations, which is most likely during the most energetically-demanding stages of reproduction and/or in the individuals that invest most in reproduction. To determine whether glucocorticoid concentrations are tightly linked to challenges associated with reproduction, studies testing the CORT-adaptation hypothesis should assess reproductive effort in addition to metrics of reproductive success. Life history stage must also be taken into account because the relationship between glucocorticoids and both reproductive effort and success can vary across life history stages (Bonier et al., 2009b; Ouyang et al., 2013, Ouyang et al., 2011).

Avian species are frequently used to address questions about reproductive effort and success because it is relatively easy to identify specific life history stages and monitor both parental effort and the reproductive outcome. In particular, the avian life history stage of incubation offers a unique opportunity to test the relationship between glucocorticoids and reproductive effort. In most avian species, an incubating bird spends its time either on the nest, regulating temperature and otherwise caring for its clutch of eggs, or away from the nest, fulfilling its own needs, often by foraging. This dichotomy in behavior means that reproductive effort can be easily quantified by measuring the time spent on or away from the nest.

To date, few studies have examined the relationship between corticosterone (the primary avian glucocorticoid) and incubation behavior, and most focus on seabird species that experience prolonged fasts during incubation. In seabirds, high corticosterone is associated with reduced effort during incubation and reproductive success. For example, both experimental and observational studies of the Adélie penguin (*Pygoscelis adeliae*) have found higher corticosterone to be associated with reduced nest attentiveness and increased likelihood of abandonment (Spée et al., 2011, Spée et al., 2010; Thierry et al., 2013). Similar patterns were found in the common eider (*Somateria mollissima*), where incubating females implanted with corticosterone were more likely to lose an egg to predation (typically due to low nest attentiveness) than control females (Criscuolo et al., 2005) and in an observational study, higher corticosterone was linked to reduced reproductive success in females that selected exposed nesting sites (D'Alba et al., 2011). However, the physiological mechanisms associated with incubation effort could be very different in species that rely on energy stores to support incubation bouts that last for hours or days (e.g. common eider [Bolduc and Guillemette, 2003], Adélie penguin [Derksen, 1977]) versus species, like most songbirds, that often incubate for less than 30 min at a time (Conway and Martin, 2000). Birds that frequently switch between incubating eggs and tending to their own needs are continuously trading off the time invested in reproduction (time on the nest) with the time invested in self-maintenance (time away from the nest). To the best of our knowledge, there have been no studies that successfully investigate the relationship between individual variation in corticosterone and incubation effort in a bird with relatively short incubation bouts (but see Lothery et al. 2014).

Here, we test the CORT-fitness hypothesis and its extension, the CORT-adaptation hypothesis in incubating female red-winged blackbirds (*Agelaius phoeniceus*), which regularly leave the nest to feed during incubation. Based on the CORT-fitness hypothesis, we predict that females with elevated circulating glucocorticoids should reduce investment in incubation and ultimately have lower reproductive success than individuals with lower glucocorticoid concentrations. Based on the CORT-adaptation hypothesis, we predict that if the energetic demands of reproduction drive variation in glucocorticoids among incubating females, females with higher corticosterone will increase their incubation effort, resulting in higher reproductive success.

2. Methods

2.1. Study species and population

Red-winged blackbirds are polygynous, and multiple females will nest on the territory of a single male (Searcy and Yasukawa, 1995). Females are the sole incubator of eggs and hatchlings (Holcomb, 1974) and are not fed by their mate (Beletsky, 1996). As a result, the relationships between a female's corticosterone, incubation behavior, and nest success might be more tightly linked than in species with bi-parental incubation or mate feeding. In red-winged blackbirds, incubation effort decreases across the incubation period (Holcomb, 1974), therefore we included day of incubation in all analyses of incubation effort. We studied red-winged blackbirds nesting across six marshes at the Queen's University Biological Station (44°34′02.3″ N, 76°19′28.4″ W) in southeastern Ontario during May–June 2014 and 2015. All marshes provided similar nesting habitat and we found no difference in the birds' corticosterone, scaled mass index, fat score, incubation effort, clutch size or mass, or hatching success across marshes (all $P > 0.25$), and thus, we do not distinguish among marshes for our analyses. All procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee and the Queen's University Animal Care Committee and were in compliance with US and Canadian national standards for the use of animals in research.

2.2. Trapping and blood collection

In 2014 and 2015, we captured 59 incubating female red-winged blackbirds in 70 capture events. We captured females between 5:30–10:30 AM on days 3–11 of the 12-day incubation period by flushing them into mist nets placed 1–3 m from the nest or by placing a single-celled walk-in (Potter) trap directly on top of the nest. Both traps and nets were set-up when the female was away from the nest. We estimated the day of incubation by counting the number of days after clutch completion or before the hatching date. Four individuals were excluded from any analyses including the day of incubation because we were unable to determine either the clutch completion date or hatch date.

Rapidly after capture, we punctured the brachial vein with a 26 gauge ½ inch needle and collected 300 μL of blood into heparinized capillary tubes. We recorded the exact time of sample collection after the bird entered the net or trap and used only samples collected within three minutes for analyses including corticosterone (Romero and Reed, 2005). We found no relationship between the log of baseline corticosterone and time from capture among the samples collected within 3 min ($\beta = 0.0004$, 95% CI $= -0.002$–0.003, $P = 0.72$, $N = 40$) or between log corticosterone and the time from net or trap set-up and capture ($\beta = 0.004$, 95% CI $= -0.01$–0.003, $P = 0.28$, $N = 28$). Therefore, we concluded that these samples were reasonable representations of baseline concentrations of corticosterone.

We fitted each individual with a unique combination of color bands and a numbered aluminum band from the Canadian Wildlife Service. We measured wing cord with a wing rule (to the nearest 1 mm), tarsus with calipers (to the nearest 0.1 mm), mass using a Pesola spring scale (to the nearest 0.5 g), and scored fat on a 0–5 scale (Wingfield and Farner, 1978). We assessed the body condition and age of females because incubation effort could be related to condition or previous breeding experience, and physiology and life history strategy could change with age. First year red-winged blackbirds have delayed plumage development such that 1 year old females have completely brown epaulet feathers (upper marginal-coverts) and females over 1 year in age have colored epaulets ranging from orange to red (Johnsen et al., 1996; Yasukawa and Searcy, 1995). Based on epaulet color, we identified females as either 1 year old (i.e., in their first breeding season) or older (i.e., in their second or later breeding season). While one person processed the female, another person used a Pesola spring scale to weigh each egg in the nest (to the nearest 0.1 g). We monitored nests every