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Egg size and yolk steroids vary across the laying order in cockatiel clutches: A strategy for reinforcing brood hierarchies?

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ABSTRACT

When a female bird begins incubation before clutch completion, the nestlings hatch sequentially, and a size hierarchy forms within the brood. This size hierarchy may be minimized or exacerbated through differential allocation of resources to eggs across the laying order. In this study, we characterize intra-clutch variation in cockatiel clutches by measuring egg mass, yolk mass, and concentrations of yolk testosterone, androstenedione, and corticosterone. Cockatiels are a long-lived member of the Psittaciformes. Because asynchronous hatching may reduce sibling competition and allow for extended development periods in long-lived birds, we predicted that female cockatiels would allocate maternal resources in a way that would reinforce the brood size hierarchy. Significant within-clutch differences in egg size and steroid concentrations were observed. Eggs at the end of the laying sequence were smaller and had significantly smaller yolks than eggs early in the laying order. Fifth-laid eggs, as well as first-laid eggs, contained significantly lower concentrations of testosterone than eggs in other positions of the laying sequence. No differences in yolk androstenedione concentration were observed. Yolk corticosterone concentrations increased linearly with laying order. Together, these patterns might reinforce the brood size hierarchy created by asynchronous hatching.

1. Introduction

In many species of birds, the sequential hatching of young produces an age and size hierarchy within the brood. Whether hatching asynchrony is a non-selected consequence of constraints on incubation (Clark and Wilson, 1981) or serves an adaptive function has been the focus of much research (reviews by Amundsen and Slagsvold (1991), Magrath (1990) and Stoleson and Beissinger (1995)). In agreement with Lack's brood reduction hypothesis (1947, 1968), hatching asynchrony is greater when food is limited (Wiebe and Bortolotti, 1994a) and increases nestling mortality under poor feeding conditions (Wiebe and Bortolotti, 1995; Rodríguez et al., 2008). Evidence also suggests that hatching asynchrony may reduce sibling competition through the formation of a stable dominance hierarchy (Hahn, 1981; Wiebe and Bortolotti, 1994b). Reduced sibling competition could promote the evolution of extended developmental periods by weakening the selection response for rapid development (Ricklefs, 1993). Prolonged embryo and nestling development, which occurs in many long-lived species, might permit offspring to allocate resources to aspects of development that would be important for maximizing lifespan (Ricklefs, 1992).

In addition to hatching order, within-clutch variation of egg size and yolk steroid concentration may also influence the growth and competitive abilities of nestling birds. Several studies suggest that nestlings hatching from larger eggs are heavier (Williams, 1994; Smith and Bruun, 1998; Styrsky et al., 1999), and may suffer lower mortality (Smith and Bruun, 1998) during the early portion of the nestling period. Maternally derived androgens and stress hormones often vary across the laying order (Groothuis et al., 2005; Love et al., 2008, 2009), and may also affect nestling growth and competitive abilities. Exposure to high concentrations of yolk androgens during embryonic development is generally correlated with increased nestling begging intensity, growth rate, and survivorship (Groothuis et al., 2005), although negative effects of yolk androgens on growth and survival have also been described (Sockman and Schwabl, 2000). Yolk corticosterone is often associated with negative phenotypic effects on offspring quality (Hayward and Wingfield, 2004; Rubolini et al., 2005; Saino et al., 2005; Love et al., 2005), but might induce phenotypes that increase offspring survival in low-quality environments. For example, nestling starlings exposed to high concentrations of yolk corticosterone fledge with more functional wing muscles and perform better on flight performance trials than controls (Chin et al., 2009). In addition, yolk corticosterone concentrations may adaptively match maternal quality with offspring demand. Poor-quality female starlings produce eggs with elevated concentrations of corticosterone. Male

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nestlings from these eggs are smaller at hatching, have depressed immunity, and increased mortality (Love et al., 2005). By facilitating brood reduction of the more costly sex, yolk corticosterone allows poor-quality females to invest less in their current offspring, and increase survival and future fecundity (Love and Williams, 2008).

The extent of hatching asynchrony differs widely and may serve different functions for different avian species. Most Passeriformes (perching or songbirds) produce clutches with limited hatching asynchrony (Ricklefs, 1993). In contrast, members of the order Psittaciformes (parrots and their relatives) often lay eggs at 2-day intervals, with incubation beginning with the first-laid egg. This results in completely asynchronous hatching. In some passerines, egg size (Cichon, 1997; Howe, 1976; Mead and Morton, 1985; Zach, 1982) and yolk androgen concentrations (Pilz et al., 2003; Schwabl, 1993) increase across the laying order. These patterns might counteract the formation of a size hierarchy and reduce the competitive disadvantages inherent to younger chicks. To date, no one study characterized yolk hormone concentrations in any psittacine bird. In this study, we measured egg mass, and yolk mass, as well as the concentrations of testosterone, androstenedione, and corticosterone within the yolks of cockatiel (*Nymphicus hollandicus*) eggs. We predicted that if asynchronous hatching were adaptive for psittacines, steroid hormones and egg components would vary in a way that could maintain the size hierarchy within the brood. Alternatively, if resources varied in a way that might minimize the consequences of the size hierarchy, this would suggest that hatching asynchrony in this species might not be directly adaptive (Love et al., 2009). We also determined whether yolk concentrations of testosterone and androstenedione correlated with serum concentrations in laying females. Few studies have found a correlation between yolk androgen concentrations and blood concentrations in females (Mazuc et al., 2003; Navara et al., 2006; Pilz and Smith, 2004; Williams et al., 2004), and it is generally assumed that yolk androgens originate in the follicle (Groothuis and Schwabl, 2008).

2. Methods

2.1. Animals and housing conditions

Cockatiels are cavity-nesting members of the order Psittaciformes that inhabit the arid interior of Australia (Forshaw, 1989). Like many species of parrot, cockatiels produce large, asynchronously hatching clutches. Eggs are laid at 2-day intervals until a clutch of approximately 4–8 eggs is obtained (Millam et al., 1996). Incubation typically begins after the first egg is laid, and lasts 18–21 days (Millam et al., 1996). Nestlings fledge at approximately 28–35 days of age (Forshaw, 1989).

The captive colony of cockatiels used in this study is maintained by the University of California, Davis. Procedures were carried out in accordance with the guidelines set by the University of California Davis Animal Care and Use Committee. Individual birds were housed in wire cages ($0.3 \times 0.3 \times 0.6$ m) arranged adjacently in 3 rows with 12 cages per row. Feed (breeder crumbles; Roudybush Inc., Sacramento, CA) and water were available continuously at one end of the cage. Pairs were first kept under a 9:15 LD photoperiod, with light onset at 8:00 AM for at least 6 weeks. Stainless steel nest boxes ($0.2 \times 0.3 \times 0.3$ m) containing pine shavings were attached to the side opposite the feed and water. Birds were exposed to 15:9 LD with light onset at 5:00 AM to encourage egg laying. Ambient temperature was approximately 20 °C.

2.2. Sample collection

Nests were monitored twice daily for laying. When an egg was discovered, it was weighed on a digital scale and marked with a permanent marker for identification. The cockatiel egg was then removed from the nest and replaced with a white plastic egg (1 in. × 0.75 in.) (plastic budgie egg, Bird Supply of New Hamphshire). All eggs were stored at −70 °C. In total, 63 eggs from 14 clutches were collected.

In order not to disrupt egg laying, blood samples were obtained from female cockatiels following the laying of a third egg. A 25-gauge needle and heparinized capillary tube were used to collect blood from the brachial vein. Blood was pooled into a serum separating tube (BD Microtainer) and centrifuged for 10 min at 2000*g*. The serum was retained and stored at −70 °C. In total, 12 samples from 12 females were collected. Blood samples were not collected from two females which laid clutches containing fewer than 3 eggs.

2.3. Yolk hormone analysis

Frozen eggs were thawed, and the yolk and albumin were separated. Yolks were then weighed, and homogenized with an equal amount of phospho-buffered saline (PBS). Hormones were extracted using absolute ethanol according to the procedure used by Kozlowski et al. (2009). Briefly, yolk samples were homogenized and incubated at 37 °C for 1 h. After incubation, 500 μL of absolute ethanol was added to 500 μL of the yolk/PBS mixture. Upon adding ethanol, the samples were immediately homogenized again, and allowed to incubate at room temperate for 10 min. Samples were then spun in a centrifuge for 10 min at 12,282*g*, and the supernatant was retained for assay.

2.4. Serum androgen analysis

Because of the limited volume of many samples, serum samples were transferred to 2 ml eppendorf tubes and diluted 1:2 with PBS. Samples were mixed with a vortexer, and an equal volume of 100% ethanol was added to precipitate proteins and lipids. Upon adding ethanol, the samples were immediately homogenized, and allowed to incubate at room temperate for 10 min. Samples were then spun at 12,282*g* in a centrifuge for 10 min. The supernatant was poured into a sterile cryule tube, and frozen at −70 °C.

2.5. Radioimmunoassays

Both yolk and serum extracts were analyzed using radioimmunoassay (RIA) in the Endocrinology Laboratory at the Saint Louis Zoo. In preparation for assay, extracts were thawed and spun in a centrifuge at 12,282*g* for 10 min to remove any remaining lipids. Testosterone, androstenedione, and corticosterone (yolk only) concentrations were measured using commercially available radioimmunoassay kits (Coat-A-Count © Testosterone 125I Kit, Coat-A-Count © Direct Androstenedione 125I Kit, Diagnostic Products Corporation, Los Angeles, CA, and DA Corticosterone kit, ICN MP Biomedicals). In the testosterone assay, the lowest detectable concentration was 0.05 ng/ml and upper concentration was 40 ng/ml. The lowest detectable concentration of the androstenedione assay was 0.04 ng/ml and upper detection concentration was 8.7 ng/ml. For corticosterone, the lower and upper detection concentrations were 0.13 and 5 ng/ml, respectively. All kits have highly specific antibodies and low cross-reactivities with other steroid hormones.

Assays were run according to kit directions, with the exception that the testosterone and androstenedione kit standards, which are supplied in human serum, were replaced by standards diluted in 10% steroid-free calf serum to reduce non-specific binding. In order to equalize the matrices of standards and samples, standard diluent was added to extracted yolk or serum samples, and steroid-free cockatiel yolk or serum extract was added to standards and quality