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Yolk androgens vary inversely to maternal androgens in Eastern Bluebirds: an experimental study

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Summary

1. Female birds deposit variable amounts of androgens in the yolks of their eggs, and it has been suggested that yolk androgen deposition is an adaptive mechanism preparing offspring for a competitive environment. Breeding pairs of Eastern Bluebirds (*Sialia sialis*) Linnaeus were stimulated with an intruder presentation while ovarian follicles were developing. Yolk steroid concentrations in eggs laid by stimulated females were then compared with yolk steroid concentrations in eggs laid by control females. Additionally, blood samples taken from a subset of control and stimulated females were analysed for plasma steroid hormone concentrations. We predicted that female bluebirds experiencing a simulated intrusion would experience increased levels of circulating plasma androgens that would be reflected by larger amounts of androgens deposited in their eggs compared with control females.

2. Patterns of steroid concentrations differed between egg yolks and female plasma. In egg yolks, androstenedione was the predominant hormone, followed by testosterone. Yolks contained minimal amounts of both corticosterone and oestradiol. In female plasma, however, corticosterone was the predominant hormone, while sex steroids were found at low levels.

3. Yolk steroid concentrations did not vary with laying order in either the control or the stimulated group, a result expected due to the relatively synchronous nature of incubation behaviour exhibited by Eastern Bluebird females.

4. Yolk androgen concentrations in eggs laid by stimulated females were significantly higher than in those laid by control females, suggesting that females increase yolk androgen deposition in response to aggressive encounters.

5. Females exposed to an intruder presentation contained significantly lower levels of plasma androgens than control females. We suggest that the deposition of androgens in the eggs serves as an adaptive method of regulating circulating androgen levels in the female, preventing potentially disruptive elevations in circulating androgen concentrations during a particularly sensitive period in the reproductive cycle.

Key-words: breeding density, maternal effects, social stress, testosterone

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Introduction

Maternal effects can have potent impacts on offspring quality and survival (Mousseau & Fox 1998). One such maternal effect – the deposition of androgens in the yolks of eggs by females of many oviparous species – has an array of physiological and behavioural effects on offspring. Specifically, high levels of yolk androgens are associated with the regulation of offspring growth (Schwabl 1996b; Eising *et al*. 2001; Groothuis & Schwabl 2002; Pilz *et al*. 2004; Navara *et al*. 2005); increases in muscle development (Lipar & Ketterson 2000); decreases in T-cell-mediated immune function (Andersson *et al*. 2004; Groothuis *et al*. 2005; Navara *et al*. 2005); and increases in both embryonic and posthatch mortality (Sockman & Schwabl 2000; Navara *et al*. 2005). It is particularly relevant to the current study that exposure to high levels of yolk androgens can result in higher begging rates in nestlings and more competitive behaviour in adulthood (Schwabl 1993). It has thus been suggested that the deposition of yolk androgens by females is an adaptive mechanism to increase levels of aggression in their offspring, to prepare offspring for a competitive environment.

In birds, concentrations of yolk androgens vary both within and among clutches. In some species in which offspring hatch asynchronously, yolk androgens are

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significantly higher in eggs laid later in the laying sequence (Schwabl 1993; Lipar & Ketterson 2000; Sockman & Schwabl 2000; Eising *et al*. 2001; French *et al*. 2001; Lipar 2001). Adaptive hypotheses propose that females deposit yolk androgens to facilitate the survival of offspring that hatch from later-laid eggs by stimulating androgen-related growth and aggressive behaviours in those offspring. Along the same lines, yolk androgen levels correlated positively with breeding density in House Sparrows (*Passer domesticus*) (Schwabl 1997); number of territorial intrusions in Tree Swallows (*Tachycineta bicolor*) (Whittingham & Schwabl 2001); relative competitive environment in Black-headed Gulls (*Larus ridibundus*) (Groothuis & Schwabl 2002); and breeding density in European Starlings (*Sturnus vulgaris*) (Pilz & Smith 2004). In each of these cases the deposition of yolk androgens was identified as a potential mechanism for stimulating offspring performance in a competitive environment.

While an increasing amount of work has focused on the effects of yolk androgens on offspring, few studies have considered the interaction between the physiological state of the female and the concentration of yolk androgens in her eggs. Many of the above-mentioned studies, in which yolk androgen levels varied with the social environment, assume the deposition of high levels of yolk androgens is directly related to high levels of circulating androgen in the female. This assumption is based on a correlational study of Common Canaries (*Serinus canaria*) in which circulating levels of plasma androgens during follicular development correlated positively to androgen levels in the eggs (Schwabl 1996a), and on studies in which experimental elevation of maternal oestradiol resulted in a corresponding increase in levels of oestradiol measured in the egg yolks of both Zebra Finches (*Taeniopygia guttata*) (Williams *et al*. 2005) and Japanese Quail (*Coturnix japonica*) (Adkins-Regan *et al*. 1995). In a study of House Sparrows, however, circulating plasma androgen levels correlated negatively with androgen concentration in the eggs (Mazuk *et al*. 2003) and in European Starlings patterns of sex steroid levels found in the plasma were different from patterns previously found in the yolks of starling eggs (Pilz & Smith 2004; Williams *et al*. 2004). These studies suggest that the relationship between androgen levels in females and their eggs may be more complicated than previously thought, and may also be species-specific. It remains unclear whether the production and deposition of high levels of androgens into eggs is associated with a concurrently high level of androgens in female circulation, or whether the egg yolk may serve as an alternative outlet for androgens produced in by the follicular cells, perhaps to avoid raising plasma androgen concentrations above basal levels.

To understand better the proximate controls of yolk androgen deposition in relation to levels of circulating androgens in the laying female, we tested experimentally the effects of both laying order and aggressive social interactions on androgen concentrations in egg yolks and female plasma in the Eastern Bluebird (*Sialia sialis*) Linnaeus. The Eastern Bluebird is an excellent study species in which to examine the effects of environmental and social interactions on both yolk and female plasma androgen levels, because individuals of both sexes are extremely aggressive and territorial during the nesting period (Gowaty 1981).

Eastern Bluebirds are socially monogamous passerines that breed over much of eastern North America. This species is an obligate secondary cavity nester (they depend on nest cavities to reproduce, but cannot excavate their own), and nest cavities are a limited resource (Gowaty & Plissner 1998). Aggressive interactions are routinely observed at nest boxes, perhaps as a protective mechanism against cavity usurpation. Thus we could manipulate the perceived competitive environment of females by presenting nesting females with a simulated intrusion by an unfamiliar female.

The effects of increased yolk androgens have been well studied in Eastern Bluebirds. Experimentally elevated testosterone levels in the eggs of Eastern Bluebirds increased posthatching growth rates in nestlings, but decreased embryonic survival and suppressed T-cell immunity (Navara *et al*. 2005). Thus the deposition of yolk androgens could serve as a method for controlling offspring quality and survival by Eastern Bluebird females.

We altered the perceived social environment of breeding pairs experimentally using an intruder presentation during the period of rapid yolk deposition, and compared the steroid hormone concentrations of the resulting eggs to those from a set of control breeding pairs. A subset of control and stimulated females were captured either immediately after stimulation or 1 day prior to the first egg date, and blood samples were taken to examine the effects of intruder presentation on circulating plasma androgen levels during the time of follicular development. We predicted that exposure of breeding females to an intruder presentation would cause an increase in circulating plasma androgens and consequently lead to an increase in the quantity of androgens in the yolks of eggs that they laid.

Materials and methods

PRESENTATION OF AN INTRUDER FEMALE

The experiment was conducted in Lee County, AL, USA on portions of a large population of Eastern Bluebirds where a majority of individuals are colour-banded under Federal License No. 21661, collecting permit No. MB784373-1 and Institutional Care and Use Committee No. 0309-R-2321. Nest boxes were checked daily for signs of nest building. Previous observations indicate that the period between completion of the nest lining and appearance of the first egg is ≈2 days (K.J.N. and L.M.S., unpublished data). Thus we timed the experiment so that intruder presentations and blood sample collections occurred on the day when the nest lining was completed. Intruder presentations were given for

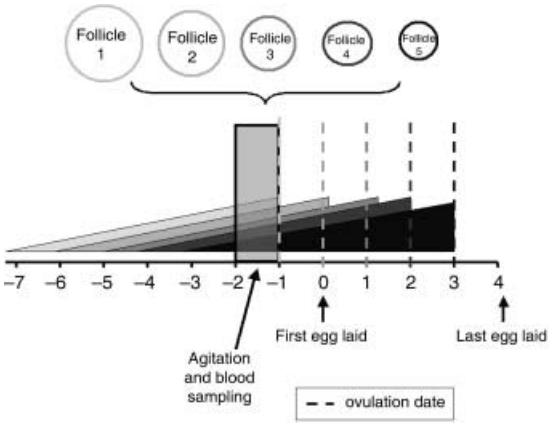

Fig. 1. Timing of intruder presentations and blood sampling of female Eastern Bluebirds in relation to phase of rapid yolk deposition for all developing follicles in a clutch. Triangles represent the 6-day period of rapid yolk deposition for each follicle; dotted lines, ovulation dates. Follicles develop at a 24-h time lapse from one another and are thus laid at a rate of one egg per day. Intruder presentation was conducted on days –2 and –3 (shaded rectangle), a time when all five follicles in the sequence were undergoing rapid yolk deposition.

30-min periods on two consecutive days, and all developing follicles in the sequence were in the stage of rapid yolk deposition during the both intruder presentations (Fig. 1). In cases where the predicted first-egg date was not correct, the timing of follicular development was determined after the first egg was laid, and eggs resulting from follicles that were not in stages of rapid yolk deposition during intruder presentation were excluded from the analyses. Despite the fact that follicles 1 and 2 were probably close to full size at the time of intruder presentation, we felt it was important to include these follicles in our analyses to be conservative, and to avoid missing variations in the timing of yolk hormone deposition within clutches.

To stimulate aggression towards female bluebirds, a captive female bluebird was presented in a cubical wire cage placed 1 m from the nest box. One of five stimulus females (captured within 2 weeks of the trial from a population located >30 miles away) was randomly assigned to each trial. All stimulus animals were active during the trials and their behaviour did not differ noticeably among different trials or among females. A tape recorder was placed beside the stimulus animal and played bluebird chatter throughout the course of intruder presentation. Control pairs were exposed briefly to the tape recorder with bluebird chatter to draw the initial attention of the pair, but were never exposed to the presence of a stimulus female.

During the entire period of intruder presentation, resident females were observed (from a blind) and several behaviours suggestive of female agitation and aggression (Plissner & Gowaty 1995) were quantified, including: (1) time between start of intruder presentation and arrival of the female at the nest box; (2) number of times the female approached and entered the nest box; (3) number of times the female attempted to attack the intruder (Gowaty 1981; Gowaty & Wagner 1988). All females displayed all the aggressive behaviours quantified during intruder presentations, suggesting that they were agitated by the presence of the intruder.

COLLECTION OF FEMALE BLOOD SAMPLES

A subset of females was captured for blood sampling and quantification of circulating plasma androgens. Stimulated females were captured using a mist net immediately after the second intruder presentation. Birds were removed from the mist net and blood was sampled promptly from the brachial vein to avoid complications associated with capture stress (Romero *et al*. 1997; Romero & Romero 2002). While exact time-frames of blood sampling were not recorded, we are confident that the time between capture and blood sampling rarely exceeded 3 min, and never exceeded 5 min. Control females were captured in mist nets 1 day prior to the appearance of the first egg. During this time a tape recorder playing bluebird chatter was used briefly to draw the attention of the nesting pair, after which the female was captured and a blood sample was taken as described above.

HORMONE ASSAYS IN PLASMA AND EGGS

Eggs were collected on the day of laying and replaced with dummy eggs to prevent nest abandonment. Because eggs had not yet been incubated, all yolk hormones measured were of maternal origin. On collection, eggs were frozen at –20 °C until the time of extraction for hormone analyses, when yolks were separated by thawing and 35 mg yolk was weighed for extraction. The procedures for extraction and radioimmunoassay of testosterone, androstenedione, 17β-oestradiol and corticosterone from yolk homogenates were as described by Schwabl (1993). Intra-assay coefficients of variation were androstenedione, 2·2; testosterone, 3·7; 17β-oestradiol, 5·6; corticosterone, 7·4%; and recovery rates were androstenedione, 66; testosterone, 53; 17β-oestradiol, 82; corticosterone, 66%. Extraction and radioimmunoassay of plasma testosterone, androstenedione, 17β-oestradiol and corticosterone were completed in one set following procedures described by Wingfield & Farner (1975); Mendonça *et al*. (1996). Recovery rates for plasma were androstenedione, 78; testosterone, 34; 17β-oestradiol, 85; corticosterone, 78%.

STATISTICAL ANALYSES

All data except for corticosterone measurements were non-normally distributed and logarithmically transformed. Parametric tests (including ANOVAs and simple regressions using STATVIEW and JMP software: SAS Institute, Cary, IN, USA, 1993) were used for all statistical analyses except for within-clutch variation in yolk hormone content, which was analysed using a nested

ANOVA using female identification as the nested factor. This allowed the inclusion of potentially important within-clutch variation, while preventing the replication that may occur using eggs within the same nest and sired by the same females as independent samples.

Results

HORMONE PROFILES IN EGGS AND PLASMA

First we examined the differences between yolk and female plasma hormone profiles, regardless of treatment group, because both overall yolk and plasma hormone profiles were similar between stimulated and control groups. None of the four yolk hormone concentrations varied significantly across the laying order (androstenedione, $F_{2,82} = 0{\cdot}61$, $P = 0{\cdot}80$; testosterone, $F_{2,81} = 0{\cdot}73$, $P = 0{\cdot}74$; 17β-oestradiol, $F_{2,83} = 2{\cdot}51$, $P = 0{\cdot}33$; corticosterone, $F_{2,83} = 3{\cdot}62$, $P = 0{\cdot}24$). Thus the respective concentrations of each hormone in egg yolks were determined using average values for each clutch. In bluebird yolks, androstenedione was the predominant hormone, followed by testosterone. Both 17β-oestradiol and corticosterone were found in relatively low concentrations in yolks (Fig. 2a). Female plasma showed a very different hormone profile: corticosterone was the predominant hormone, while androstenedione, testosterone and 17β-oestradiol were at relatively low levels (Fig. 2b).

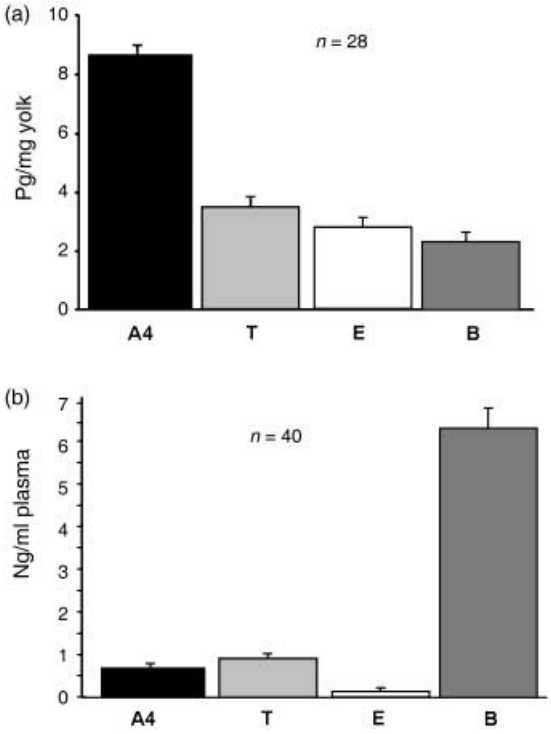

Fig. 2. Hormone profiles including androstenedione (A4); testosterone (T); oestradiol (E); corticosterone (B) found in (a) Eastern Bluebird yolks (average clutch values); (b) female circulating plasma (± SE) collected during the period of rapid yolk deposition and follicular development. *n*, Number of (a) clutches; (b) females included in the analyses.

BEHAVIOURAL RESPONSES TO INTRUDER PRESENTATION

Twenty-eight females were exposed to intruder presentations and 20 were assigned to the control group. All but one female arrived at the intruder site within minutes of the intruder presentations. This female did not arrive at the box during the entire duration of the first intruder presentation and was excluded from all analyses. All remaining females arrived at the nest box within a mean of 1·5 min of trial initiation, checked the contents of their nest boxes repeatedly (mean = 13 times per trial), and attacked the caged intruder (mean = 12 attacks per trial). Over the course of the experiment some nests were lost to predators and are not included in these analyses.

YOLK HORMONE LEVELS IN RESPONSE TO INTRUDER CHALLENGE

Presentation of an intruder to nesting females resulted in a significant increase in levels of androstenedione and testosterone in yolks compared with controls (androstenedione, $F_{2,26} = 17{\cdot}89$, $P < 0{\cdot}001$; testosterone, $F_{2,26} = 15{\cdot}42$, $P < 0{\cdot}001$) (Fig. 3). Levels of 17β-oestradiol and corticosterone in yolks did not differ with intruder presentations (17β-oestradiol, $F_{2,26} = 1{\cdot}04$, $P = 0{\cdot}32$;

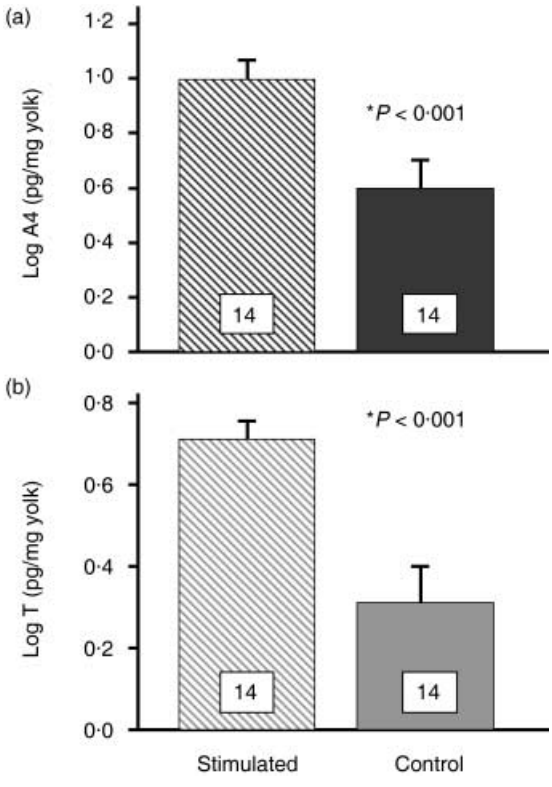

Fig. 3. Log yolk (a) androstenedione (A4); (b) testosterone (T) (± SE) values for eggs laid by stimulated and control females. Hatched bars, eggs in stimulated groups; solid bars, eggs in control groups. Clutch means were used for these analyses. Values in bars, number of clutch means in the analysis. Actual mean hormone values: androstenedione, stimulated, 10·99; control, 5·44; testosterone, stimulated, 5·17; control, 2·63 pg mg^{-1}.

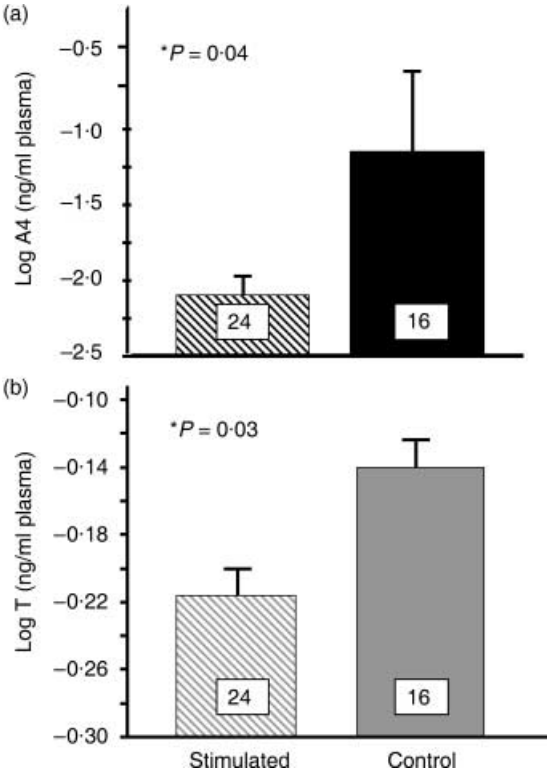

Fig. 4. Log female plasma (a) androstenedione (A4); (b) testosterone (T) (± SE) values for stimulated (hatched bars) and control (solid bars) females captured during experiment 2. Values in bars, number of females in the analyses. Actual mean hormone values: androstenedione, stimulated, 0·045; control, 0·364; testosterone, stimulated, 0·604; control, 1·433 ng mg^{-1}.

corticosterone, $F_{2,26} = 1{\cdot}18$, $P = 0{\cdot}29$). Although females showed variation in aggressive behavioural responses to intruding females (time to respond to intruder; number of nest checks; number of attacks on intruder), simple regressions showed no significant relationship between aggressive behaviour and levels of any of the yolk androgens (for all hormones: time to respond, $R^2 < 0{\cdot}15$, $P > 0{\cdot}10$; number of nest checks, $R^2 < 0{\cdot}12$, $P > 0{\cdot}15$; number of attacks, $R^2 < 0{\cdot}27$, $P > 0{\cdot}15$).

FEMALE PLASMA HORMONE LEVELS IN RESPONSE TO INTRUDER CHALLENGE

The presentation of an intruder had a significant effect on levels of circulating androstenedione and testosterone in females: both hormones were significantly lower in stimulated females compared with controls (androstenedione, $F_{1,37} = 4{\cdot}37$, $P = 0{\cdot}04$; testosterone, $F_{1,37} = 5{\cdot}11$, $P = 0{\cdot}03$) (Fig. 4). However, levels of 17β-oestradiol and corticosterone circulating in female plasma did not differ between stimulated and control females (17β-oestradiol, $F_{1,37} = 0{\cdot}40$, $P = 0{\cdot}53$; corticosterone, $F_{1,37} = 0{\cdot}03$, $P = 0{\cdot}86$). Again simple regressions showed that none of the four hormones varied in concentration with degree of aggressive behaviour (for all hormones: time to respond, $R^2 < 0{\cdot}04$, $P > 0{\cdot}60$, number of nest checks, $R^2 < 0{\cdot}04$, $P > 0{\cdot}61$; number of attacks, $R^2 < 0{\cdot}33$, $P > 0{\cdot}10$).

Discussion

Hormone profiles and concentrations in both egg yolks and female plasma were first examined regardless of treatment group to determine if hormone levels in the yolk vary across the laying order, and how yolk hormone levels relate to circulating hormone levels in female plasma at the time of follicular development. Our study is one of the few to sample hormone levels circulating in female plasma during the critical timeframe of rapid yolk deposition (but cf. Schwabl 1996a; Williams *et al*. 2005). All four hormones measured (androstenedione, testosterone, 17β-oestradiol and corticosterone) were detected in the yolks of Eastern Bluebird eggs. Androstenedione was the most prominent androgen in Eastern Bluebird eggs as was also the case with the eggs of Common Canaries (Schwabl 1993); American Kestrels (*Falco sparverius*) (Sockman & Schwabl 2000); and Black-headed Gulls (Groothuis & Schwabl 2002). In contrast, testosterone was the predominant androgen in the eggs of both Zebra Finches (Gil *et al*. 1999) and House Finches (*Carpodacus mexicanus*) (Navara *et al*. 2006). Yolk hormone concentrations did not vary according to laying order. The relative constancy of levels of yolk androgens across the laying sequence is not surprising given that bluebird eggs hatch more synchronously and offspring exhibit less of a size gradient compared with other passerine species.

Hormone profiles found in the plasma of females at the time of follicular production were substantially different from those of eggs that were being yolked at that time. Corticosterone was the most prominent hormone in female plasma, while androstenedione, testosterone and 17β-oestradiol were found in relatively low levels. These data demonstrate that yolk hormone content and circulating hormone concentrations in female plasma are not directly proportional in Eastern Bluebirds, despite the demonstration of a link between circulating hormone levels and yolk hormone concentrations in other species (Love *et al*. 2005; Williams *et al*. 2005). Our data are instead consistent with a study on Japanese Quail in which the authors concluded that >99% of yolk steroids come directly from the cells of the follicular wall, rather than from female circulation (Hackl *et al*. 2003), suggesting that hormones produced in follicular cells may never reach circulation in the female bird. Further evidence of the disconnection between yolk hormone content and circulation plasma hormone content in the female is seen in the results of our intruder presentation experiment. Yolk androgen concentrations were significantly higher in eggs yolked during an intruder presentation compared with controls, suggesting that the presence of an intruder female stimulates an increase in the deposition of androgens into eggs. Yet plasma androgen levels in stimulated females were significantly lower than in control females.

Our study is the first to experimentally demonstrate changes in concentrations of androgens in yolk and

female plasma in response to an aggressive challenge. A similar experimental test, in which a male intruder was presented to nesting House Sparrow pairs, did not show a change in yolk androgens or in female plasma androgens in response to the intrusion (Mazuk *et al*. 2003). In the House Sparrow study, however, the intruder was male, with little threat of territorial invasion for a female bird, and was presented for 5-h periods, which may greatly exceed the duration of a normal aggressive interaction between birds. In such an extended period, nesting pairs may have dismissed the intruder as presenting no threat, in which case females may not alter deposition patterns of yolk androgens. Additionally, female blood samples were taken after clutch completion, long after the critical time during which hormone levels would have changed in response to the intruder presentation, and during a time when androgen levels are known to decrease with the onset of incubation (Wingfield *et al*. 2001).

A large body of previous work has suggested a role for androgens in aggressive behaviour, which may be associated in both males and females with a rise in circulating testosterone levels (Petrie 1983; Wingfield *et al*. 1987; Silverin 1990; Staub & De Beer 1997; Eens & Pinxten 2000; Nelson 2000). Neither circulating androgen levels in females nor yolk androgen content was significantly correlated with any of our measures of aggressive behaviour. Perhaps all females in the study were maximally agitated by the presence of an aggressive intruder, eliminating the hormonal variation we might see if the intensity or duration of the intrusion varied. Additionally, contrary to predictions, female bluebirds that experienced a simulated aggressive interaction had significantly lower levels of androstenedione and testosterone than control females. These results are consistent with the findings of Elekonich & Wingfield (2000), who showed that plasma testosterone levels are significantly lower in female Song Sparrows (*Melospize melodia*) experiencing aggressive interactions, compared with control females. Similarly, Cristol & Johnsen (1994) showed that testosterone levels in Red-winged Blackbirds (*Agelaius phoeniceus*) decrease significantly before the annual period of aggressive and territorial behaviour ends; they proposed that this decrease in testosterone levels is adaptive, protecting against potential interruptions in the reproductive cycle that can result from high testosterone levels. Elevated androgen levels have been shown to interrupt processes associated with ovulation and reproductive cyclicity in both birds and mammals (Harper 1969; Searcy 1988). Additionally, aggressive behaviour associated with an increase in testosterone may interrupt behaviour necessary for a successful breeding attempt (Hegner & Wingfield 1987; Oring *et al*. 1989; Wingfield *et al*. 2001).

We suggest that the deposition of androgens into the eggs serves as an adaptive method of regulating circulating androgen levels in the female. Such a regulatory method could help females to maintain androgens circulating in the plasma at basal levels, preventing androgen-driven interruptions of the reproductive cycle during aggressive interactions. As such, eggs act as a 'sink', absorbing androgens produced by follicular cells in response to aggressive interactions before the sex steroids are secreted into circulation. Such a phenomenon has been proposed for the protective isolation of pollutants in eggs: fish, amphibian and avian eggs may act as excretion sites for pollutants, protecting females from the potentially harmful, disruptive effects associated with exposure to pollution (Kleinow *et al*. 1999). Thus it is not hard to imagine that a similar excretion method may exist for potentially disruptive hormones.

While the potential mechanisms behind such an adaptive deposition strategy are still unknown, mounting evidence suggests that female birds do adjust yolk androgen content in response to social and environmental stimuli (Schwabl 1997; Whittingham & Schwabl 2001; Groothuis & Schwabl 2002; Pilz & Smith 2004; Navara *et al*. 2006). These findings suggest the existence of a regulatory mechanism behind the deposition of yolk androgens. The use of developing follicles as an alternative for androgens that are produced naturally in response to social encounters could provide a female with a quick way of controlling her hormonal milieu, without the slower, more permanent effects of altering steroid receptor numbers within her own body, a mechanism that may be harder to vary seasonally.

Our hypothesis is further supported by the different profiles of sex and adrenal steroid hormones in bluebird plasma and egg yolks. Circulating levels of sex steroids in female plasma were relatively low, perhaps resulting from the shuttling of those steroids into the yolks of developing follicles, while corticosterone was found in relatively higher concentrations within female circulation. Given that sex steroids are produced by follicular cells surrounding the developing oocyte, and corticosterone is produced at an entirely different location within the body (the adrenal glands), it is likely that the androgen content of yolk is representative of steroids that never made it into circulation, perhaps because of active shuttling across the oocyte plasma membrane.

An increasing body of work addresses the question of the adaptive significance of yolk androgens by examining the effects of high doses of androgens on developing offspring. Previous work has shown that high levels of yolk testosterone have significant effects on the size, immunocompetence and embryonic survival of Eastern Bluebird nestlings (Navara *et al*. 2005), suggesting that yolk androgens may be adaptive tools utilized by female Eastern Bluebirds as a means of altering offspring quality and reproductive success. The current study also questions the significance of how yolk androgens may alter the female's physiological state, and is the first to identify the deposition of yolk androgens as a potentially protective mechanism against the disruptive effects of androgens during a sensitive period in the reproductive cycle. One might question why, if the deposition of androgens into eggs is a protective mechanism, control females did not also deposit

maximal amounts of androgens into their eggs. Presumably, the ability to deposit androgens in variable amounts has arisen through a combination of physiological constraints on both female and offspring. For example, the deposition of high androgen concentrations into eggs has been shown to suppress the immune system in offspring (Groothuis *et al*. 2005; Navara *et al*. 2005). However, if the benefits of protecting an entire reproductive attempt from disruption by a rise in plasma androgens outweighs the immunological detriments experienced by offspring after exposure to high yolk androgen levels, the deposition of androgens into eggs after an aggressive encounter would be selected for. Additionally, in situations when offspring are not faced with a multitude of pathogens, the deposition of androgens into eggs could represent a solely beneficial influence on offspring by increasing growth rates, and may be adaptive for both female and offspring. There is a need for more experimental studies before we can fully understand the adaptive significance of, and the constraints associated with, the deposition of yolk androgens.

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