Variable Effects of Yolk Androgens on Growth, Survival, and Immunity in Eastern Bluebird Nestlings

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ABSTRACT

Female birds allocate androgens differentially within and among clutches, and it has been suggested that this is a strategy to maximize reproductive success. Only a few studies, however, have examined the effects of yolk testosterone (T) on the growth and development of nestlings, and none have reported on the immunological effects of yolk T nor have they examined several different effects in the same nestlings. To examine the effects of yolk T on nestling eastern bluebirds, we administered two doses of exogenous T to bluebird eggs and measured the growth and immunological responsiveness in the resulting nestlings. We found that yolk T is detrimental to developing embryos, with hatching success decreasing with increasing doses of yolk T. Moderate doses of yolk T stimulated skeletal growth during the embryonic period, while high doses of yolk T resulted in nestlings that weighed more and were more mature at fledging but had a compromised T-cell immune response to phytohemagglutinin. These data suggest that the alteration of reproductive success through the allocation of yolk T is a complicated phenomenon that involves the integration of several physiological effects.

Introduction

Maternal investment can have a profound effect on the growth, development, and ultimately the fitness of offspring. Many behavioral measures of maternal investment, such as incubation, provisioning, and the protection of offspring have been well studied; however, female birds have the ability to invest even more directly in their offspring by altering the hormone content of the yolk of their eggs (Schwabl 1993; Janzen et al. 1998; Lovern and Wade 2001).

It has recently been shown that androgens such as testosterone (T), dihydrotestosterone, and androstenedione are available to embryos in the yolks of avian eggs (Schwabl 1993). In many species of birds, yolk androgen concentrations vary within and between clutches, and it has been hypothesized that they can have major effects on the developing offspring (Winkler 1993; Schwabl 1996, 1997*b*; Lipar and Ketterson 2000; Sockman and Schwabl 2000; Reed and Vleck 2001). Attempts to understand the differential allocation of yolk hormones have focused on environmental and social contexts in relation to yolk hormonal content. For example, several studies have examined patterns of yolk androgen deposition in relation to breeding condition. In the American coot (*Fulica americana*), black-headed gull (*Larus ridibundus*), and house sparrow (*Passer domesticus*), females deposit more yolk androgens when breeding under more crowded conditions (Schwabl 1997*a*; Reed and Vleck 2001; Groothuis and Schwabl 2002) and when the female experiences more aggressive interactions (Whittingham and Schwabl 2002). Additionally, female zebra finches (*Taeniopygia guttata*) deposit more androgens into yolk when mated to a more attractive male (Gil et al. 1999). Females of some species also adjust the amount of hormone allocated to eggs in different positions in the laying sequence, typically allocating more androgens into later eggs (Schwabl 1993; Lipar et al. 1995; French et al. 2001; Royle et al. 2001; Sockman et al. 2001; Groothuis and Schwabl 2002). Because androgens have been known to increase aggression (Ketterson et al. 1992) and begging behavior (Schwabl and Lipar 2002), chicks exposed to more yolk androgens may do better in competition with siblings. In this way, females may be able to compensate for the offspring size gradient caused by hatching asynchrony and potentially increase their reproductive success (Schwabl 1993; Schwabl et al. 1997).

Although these results suggest a strategy behind the allocation of yolk androgens, there remains little detailed information on the effects of yolk androgens on the development of songbirds. Steroid hormones deposited into the yolk by female birds and reptiles have been found to have a mélange of positive and negative effects on offspring fitness. Injections of androgens into the yolks of canary eggs (*Serinus canaria*) increased begging behavior and growth rates of chicks (Schwabl 1996), and yolk T levels were positively correlated with growth of the hatching muscle in red-winged blackbirds (*Agelaius phoeniceus*; Lipar and Ketterson 2000). However, injections of androgens into the

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yolks of American kestrel (*Falco sparverius*) eggs resulted in offspring that hatched later and had a higher mortality rate (Sockman and Schwabl 2000).

The current literature tends to characterize the allocation of androgens to the yolk as either physiologically beneficial or detrimental. There is growing evidence, however, that T in the yolk has complex and often conflicting physiological effects. (Wingfield et al. 2001). In addition to the documented effects on young birds of yolk androgens, in adults, T increases song rate, aggression, nest defense, home range sizes, and mating success (Nolan et al. 1992; Ketterson and Nolan 1999) but also decreases overwinter survival (Nolan et al. 1992) and has immunosuppressive effects (Olsen and Kovacs 1996; Casto et al. 2001; Duckworth et al. 2001). Yolk androgens may have similar types of effects in developing nestlings as well. Thus, it seems more instructive to view yolk T as a mediator of developmental events, affecting partitioning of resources between different demands such as the energetic demands of hatching, growth, competition for food, and immunological defenses. The optimal dose of yolk androgens provided by the female will therefore be dependent on the integration of many androgenic effects within the body. Although offspring exposed to high levels of yolk androgens will experience both positive and negative physiological consequences, the environmental and social conditions in which the female is producing offspring and in which the offspring develop in the first days after hatching presumably will determine the optimal level of T to be allocated. Thus, rather than discussing the adaptive significance of a single optimum allocation strategy, we should think in terms of a range of adaptive responses to a range of environmental challenges.

To test the effects of T on a developing songbird, we injected the eggs of wild eastern bluebirds (*Sialia sialis*) with one of three treatments: a high dose of T (high dose), a low dose of T (low dose), or a control injection (control). We measured the hatching success of eggs and growth of resulting nestlings every 3 d until fledging. All nestlings were also subjected to challenges with phytohemagglutinin (PHA) to measure cell-mediated immune response and sheep red blood cells (SRBCs) to measure humoral immune response. Finally, we measured differential white blood cell (WBC) counts. In this way, we monitored a variety of developmental and physiological parameters that may be affected by yolk T. On the basis of previously published observations of other songbird species, we hypothesized that T would have an overall stimulatory effect on nestling growth while having a suppressive effect on nestling immunocompetence. To our knowledge, this is the first study to consider both the growth and immunological consequences of yolk androgen allocation in the same individuals.

Material and Methods

The eastern bluebird is a socially monogamous, sexually dichromatic passerine. During the spring of 2003, we monitored 115 eastern bluebird nest boxes located in Lee County, Alabama, for signs of nest building and egg laying. The treatment of nestlings was approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2003-0466).

Egg Injections

Immediately after the completion of each clutch, all eggs within that clutch were assigned to one of three treatment groups and injected with one of the following: (1) 3 μg T in 5 μL peanut oil (high dose), (2) 0.3 μg T in 5 μL peanut oil (low dose), or (3) 5 μL peanut oil (control). These injection amounts were based on yolk levels found in bluebird eggs collected from the study site in the previous year, which were found to vary from 2.9 ng/yolk to 240 ng/yolk (K. J. Navara, unpublished data). These injection amounts were slightly above physiological levels to compensate for degradation or incomplete incorporation of the hormone into the yolk. Clutches were assigned at random to one of the three treatment groups. Treatment was injected into the small end of the egg with a 5-μL Hamilton syringe before the onset of embryonic development.

To test whether the vehicle containing the treatments actually reached the yolk, we injected 5 μL of peanut oil stained with Sudan B into two eggs and retrieved them after 2 d. Yolks were frozen and separated from the albumin. Yolks of all injected eggs contained a homogeneous amount of blue dye throughout (albumin contained none), suggesting that the treatments do, in fact, diffuse uniformly into the yolk within two days' time. Further, previous poultry studies have successfully used this egg injection method for in ovo androgen manipulations (Henry and Burke 1999).

Nestling Growth Measurements

Nestlings hatching from treated eggs were measured on days 2, 5, 8, and 14 posthatch. Morphological measurements taken on each of these days included mass using a 30-g spring scale (accuracy = 0.2 g), right tarsus length, right wing length, and bill length using manual dial calipers (accuracy = 0.01 mm). Using the residuals of body mass and tarsus length, we calculated the condition index as body mass : tarsus length ratio, which is often used as a measure of condition in avian nestlings (Richner et al. 1993; Yom-Tov 2001). Additionally, we used the residuals of body mass and wing length to calculate maturity as body mass : wing length ratio, a measure that is often used in avian nestlings (Hario 2001). On day 14, we were also able to assess the sex of many of the nestlings because of the development of sexually dimorphic color patterns.

Nestling Hormone Levels

On day 10, the first day posthatch when a sufficient blood sample could be obtained, 60 μL of blood were taken from the

brachial vein of nestlings with a 26-gauge needle, and plasma was separated through centrifugation. Steroid hormones were isolated from plasma using liquid column chromatography according to Schwabl (1993) and quantified using one radioimmunoassay as described in Mendonça et al. (1996). Lower detection limit of this assay is 10 pg. Average recoveries for T after extraction and separation by column chromatography were 54% and intra-assay variation was 3.3%.

Cell-Mediated Immunity

PHA is a known T-cell stimulant in passerine birds (Goto et al. 1978). Injection of this antigen results in swelling around the injection site within 24 h. On day 15 posthatch, a 1-cm patch on the left midpatagium was cleared of feathers. Two measures of thickness were taken using a pressure-sensitive digital micrometer (accuracy = 0.05 mm). The bare skin was swabbed with alcohol, and 20 μg of PHA in 50 μL phosphate-buffered saline (PBS) was injected subcutaneously using a 27-gauge needle. As a control, 50 μL of PBS was injected into the right patagium. Injection dosages were extrapolated according to weight from the amounts used in a variety of passerine species in a study by Smits and Williams (1999). Two measurements of wing web thickness were taken after 24 h to assess swelling. A PHA index was computed according to Fair and Myers (2002) as the thickness of the PHA-inoculated wing web minus the thickness of the control-inoculated wing web, standardized by the average patagium thickness before inoculation:

$$\text{PHA index} = \frac{\text{post-PHA} - \text{post-PBS}}{(\text{pre-PBS} + \text{pre-PHA})/2}.$$

This formula requires that left and right wing webs do not differ in preinjection thickness, which in fact is the case here ($df = 43$, $t = -0.271$, $P = 0.78$). The PHA index was indicative of the T-cell responsiveness and thus of cell-mediated immunocompetence.

Humoral Immunity

To assess the humoral immune response, all chicks were inoculated intraperitoneally on day 5 posthatch with 0.2 mL of a 10% SRBCs (Colorado Serum, Denver) in PBS. Cells were washed twice in PBS and resuspended to the desired concentration. Antibodies produced in response to the SRBCs were quantified after 10 d (on day 15 posthatch) by taking 50 μL of blood from the brachial vein and performing a standard hemagglutination assay (Hay and Hudson 1989). In short, 20 μL of plasma were serially diluted in 20 μL of PBS (1 : 2 to 1 : 1,024) in 96-well V-bottom plates. Wells 11 and 12 served as negative and positive controls and did not contain any plasma. Instead, these wells were loaded with 20 μL of PBS or 20 μL of antisheep hemolysin (Colorado Serum), respectively. Next, 20 μL of a 2% SRBC suspension in PBS were added to each well. The plates were incubated at room temperature for 24 h. Finally, wells containing plasma samples were compared with positive and negative controls. Antibody titers were expressed as the $\log_2$ of the highest dilution of plasma containing hemagglutination (Lochmiller et al. 1993). Because the skin covering the abdomen is translucent in bluebird nestlings, it was easy to confirm visually the correct injection location.

Differential WBC Counts

A blood smear was made on day 15 posthatch from the blood sample taken for the hormone analysis. These smears were stained with Wright-Giemsa stain, and 100 WBCs were classified according to Dein (1986), after which leukocyte percentages were calculated and differentially grouped as lymphocytes, heterophils, and eosinophils. The heterophil : lymphocyte ratio, often used as an overall measure of immunity, was also calculated (Fair and Myers 2002).

Statistical Analysis

Differences in hatching success between groups were analyzed using a χ^2 test that included the number of eggs that hatched (surviving nestlings) and the number of eggs that did not hatch (indicative of embryonic mortality) in each treatment group. Measures of nestling growth, including wing length, tarsus length, bill length, and body mass, were analyzed using principal components analysis. Resulting principal components were compared using an ANOVA. Post hoc comparisons were used to examine differences between individual treatment groups. The effects of treatment on swelling response to PHA were analyzed using an ANOVA. Individual leukocyte percentages were arcsine transformed and analyzed using an ANOVA. To control for the potential effects of brood size in this experiment, we initially used brood size as a covariate in all analyses, but because the size of the brood did not contribute significantly to the variation in any measure of size or immunocompetence, it was eliminated as a covariate in all analyses ($P > 0.16$ in all cases). Additionally, because our experimental design does not control for genetic contributions to the growth and immunocompetence of these offspring, brood averages were taken for each measurement rather than treating nestlings as individuals in these analyses.

Results

Hatching Success

Injection of treatments had a significant effect on hatch rate. Eggs that were injected with the control treatment displayed a significantly lower hatching success than a separate set of eggs that were not injected ($\chi^2 = 13.23$, $P < 0.001$), illustrating a

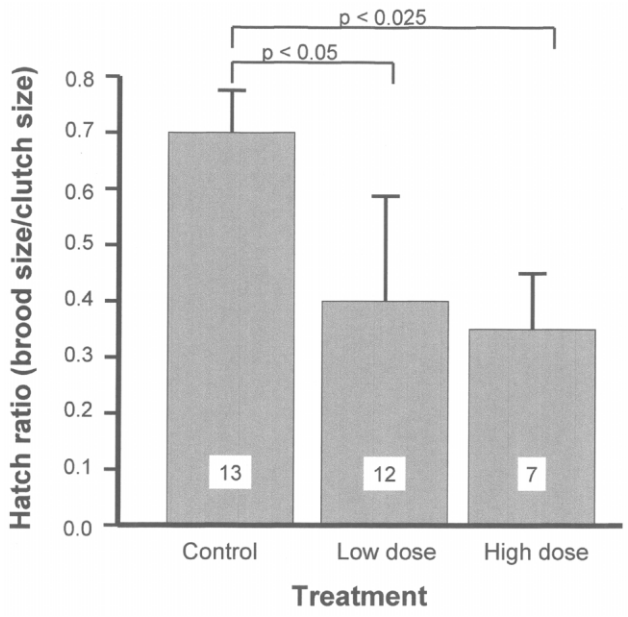

Figure 1. The effects of in ovo testosterone and control injection treatments on mean (±SE) hatch rate of eastern bluebird nestlings. Hatch rate was defined as the mean number of nestlings hatching per nest for each treatment. Clutch size was controlled for using an ANCOVA. Injection treatments included a high-dose injection (3 μg T in 5 μL peanut oil), a low-dose injection (0.3 μg T in 5 μL peanut oil), and a control injection (5 μL peanut oil). The number located in each bar indicates the number of clutches included in the analysis.

detrimental effect of the injection itself on the developing embryo.

The χ^2 tests between eggs from the three injection treatments illustrated that treatment of eggs with both a low dose of T and a high dose of T significantly decreased hatching success over controls (low dose: $\chi^2 = 4.06$, $P < 0.05$; high dose: $\chi^2 = 5.38$, $P < 0.025$; Fig. 1). All eggs that did not hatch were checked for signs of bacterial infection (a dark mass surrounding the injection site on the inside of the egg) resulting from the injection itself, and only those without bacterial infection were retained in the analysis.

Nestling Growth and Nestling Hormone Levels

For the three measures of skeletal growth, including right tarsus length, right wing length, and bill length, one principal component, PC1, explained 88.5% of the variance in size on day 2 posthatch, 84.4% on day 5, 61.3% on day 8, and 52.4% on day 14. Therefore, PC1 was used as a measure of skeletal size at all stages.

On day 2 posthatch, there was no overall difference in PC1 among treatment groups ($F = 2.00$, df = 2,12, $P = 0.18$); however, unpaired *t*-tests indicated that the PC1 of low-dose nestlings approached a significant increase over controls ($P = 0.07$; Fig. 2). Nestling weight did not differ among the treatments at this stage ($F = 0.76$, df = 2,12, $P = 0.48$). Size differences among treatment groups disappeared by day 5, and there were no differences in any measure of size among the treatment groups for days 5 and 8 posthatch (day 5: PC1, $F = 0.425$, df = 2,18, $P = 0.66$; mass, $F = 0.442$, df = 2,18, $P = 0.65$; day 8: PC1, $F = 0.103$, df = 2,19, $P = 0.90$; mass, $F = 0.184$, df = 2,19, $P = 0.83$). At day 14, however, weight differed significantly among the treatment groups ($F = 5.108$, df = 2,18, $P = 0.018$). Post hoc comparisons indicated that high-dose nestlings were significantly heavier than nestlings in the control ($P = 0.013$) and low-dose ($P = 0.008$) treatment groups (Fig. 3). Skeletal size, as measured by PC1, did not differ among nestlings in the different treatments at this stage of development ($F = 1.127$, df = 2,19, $P = 0.34$).

Our measure of condition (residuals of body mass : tarsus ratio) on day 14, just before fledging, varied significantly among treatment groups ($F = 5.88$, df = 2,18, $P = 0.01$). Post hoc comparisons indicated that the condition index of chicks in the high-dose treatment group was significantly larger than nestlings in the control ($P = 0.009$) and low-dose ($P = 0.005$) treatment groups (Fig. 4). Additionally, our measure of maturity (residuals of body mass : tarsus ratio) varied significantly among treatment groups on day 14 ($F = 6.38$, df = 2,18, $P = 0.008$). Post hoc comparisons indicated that chicks from the high-dose treatment group were significantly more mature than those in the control ($P = 0.004$) and low-dose ($P = 0.008$) treatment groups (Fig. 5).

On day 10 posthatch, T levels did not vary among the treatment groups ($F = 0.513$, df = 2,15, $P = 0.61$).

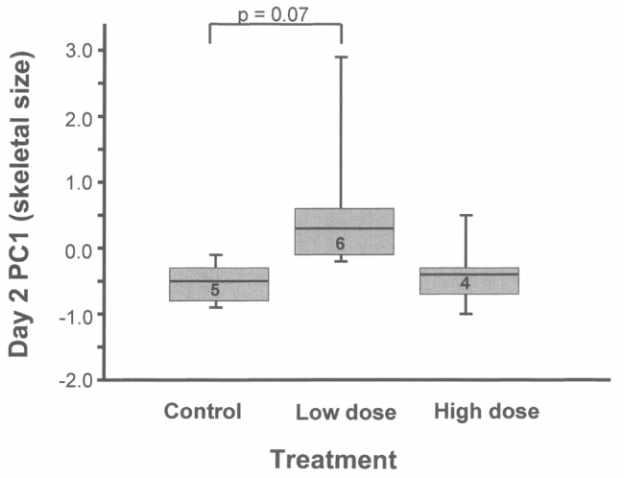

Figure 2. The effects of in ovo testosterone and control injection treatments on mean (±SE) PC1, a principal component of skeletal growth (including right tarsus length, right wing length, and bill length), on day 2 posthatch. Injection treatments are the same as those described in Figure 1.

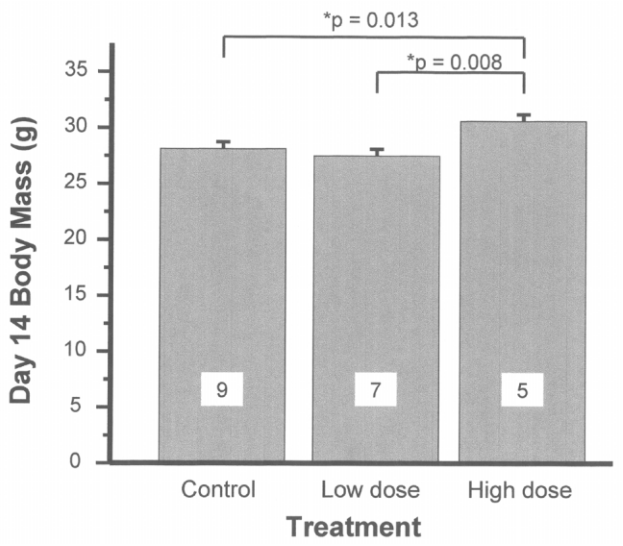

Figure 3. The effects of in ovo testosterone and control injection treatments on mean (±SE) body mass on day 14 posthatch. Injection treatments are the same as those described in Figure 1.

Nestling Cell-Mediated Immunity

There was a significant difference in swelling response among the treatment groups ($F = 3.15$, df $= 2,30$, $P = 0.03$). Nestlings hatching from eggs injected with a high dose of T had a significantly smaller swelling response to PHA than controls ($P = 0.01$). Low-dose nestlings had a smaller swelling response than controls, but this difference was not significant ($P = 0.08$; Fig. 6).

Nestling Humoral Immunity

The results of the hemagglutination titration assays on plasma from days 10 and 15 posthatch showed a lack of a humoral immune response, and further tests could not be done because nestlings had fledged. On day 10, only five chicks out of 64 showed a positive antibody response, all of which had a titer of 1 out of a possible 10. Although all of the antibody-positive chicks hatched from control eggs, this number of responding nestlings is too small for any conclusions to be drawn. Further, on day 15, only two chicks showed a positive antibody response, both of which had a titer of 1, and these were not the same chicks that showed a positive response on day 10.

Differential WBC Counts

Percentages of all leukocyte types were similar among treatment groups (heterophils: $F = 1.35$, df $= 2,67$, $P = 0.27$; lymphocytes: $F = 1.22$, df $= 2,67$, $P = 0.30$; basophils: $F = 0.51$, df $= 2,67$, $P = 0.60$; eosinophils: $F = 0.985$, df $= 2,67$, $P = 0.38$; monocytes: $F = 0.03$, df $= 2,67$, $P = 0.97$). Additionally, the heterophil : lymphocyte ratio was similar among treatment groups as well ($F = 0.179$, df $= 2,67$, $P = 0.84$).

Discussion

We observed that yolk androgens have a variety of effects on the growth and development of young bluebirds, some potentially positive, others potentially negative. Hatching success significantly decreased with both a low and a high dose of yolk T. This observation is not surprising given that T has been shown to cause developmental arrest of embryonic crustaceans (Mu and LeBlanc 2002) and is associated with higher levels of apoptosis in human vascular endothelial cells (Ling et al. 2002). Additionally, T has been shown to induce directly oxidative stress in many tissues (von Schantz et al. 1999), which could retard embryonic growth. The low survival of high-dose nestlings through the embryonic period could result from one or more of these effects.

Low doses of yolk T had a stimulatory effect on skeletal growth during the embryonic period, resulting in larger offspring at hatch. Although this difference at hatch was not significant ($P = 0.07$), when we used nestlings as individuals in our analysis (instead of using brood averages of size measures), nestlings receiving a low dose were significantly larger than control nestlings ($P = 0.02$).

The fact that offspring in the low-dose treatment group tended to have larger measures of skeletal size (PC1) on day 2 than offspring in the other two treatment groups is interesting because it suggests that yolk T in moderate amounts has a stimulatory effect on embryonic growth of the resulting off-

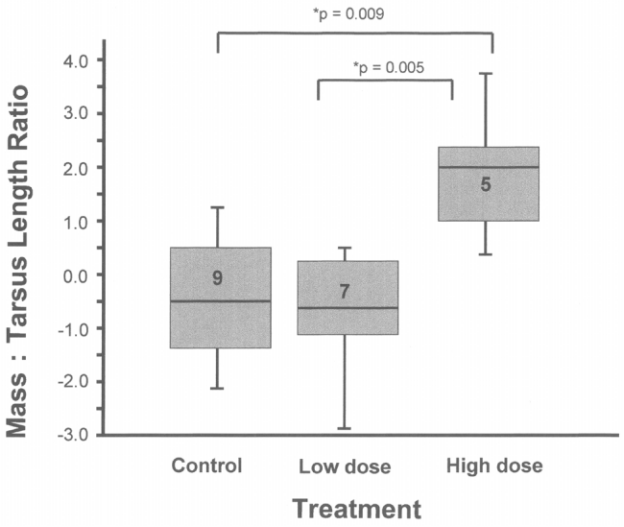

Figure 4. The effects of in ovo and control injection treatments on mean (±SE) condition (measured as the ratio of the residuals of body mass : tarsus length). Injection treatments are the same as those described in Figure 1.

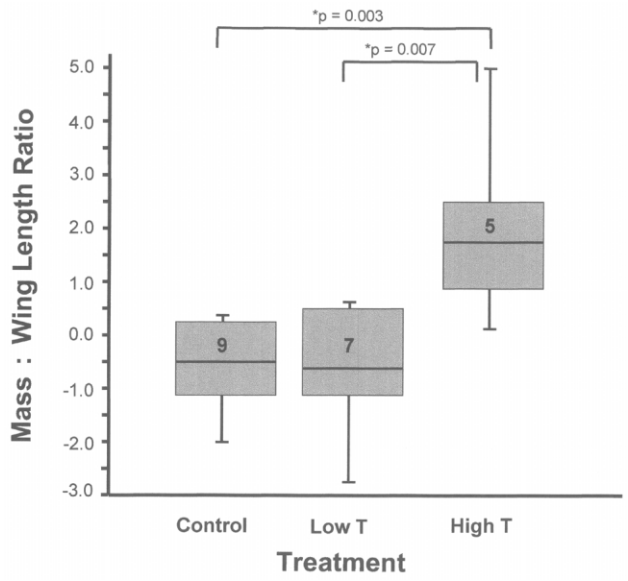

Figure 5. The effects of in ovo testosterone and control treatments on mean (±SE) maturity (measured as the ratio of the residuals of body mass : wing length). Injection treatments are the same as those described in Figure 1.

spring. These results are consistent mechanistic studies of the effects of androgens on the growth of several body tissues. For example, bone and cartilage contain androgen receptors, making them androgen target tissues (Corvol et al. 1992). Androgens have been found to stimulate the release of bone growth factors (Kasperk et al. 1990) as well as cartilage cell proliferation (Fischer et al. 1995). Thus, it is not surprising that in ovo T injections had a stimulatory effect on skeletal growth during the embryonic period. All differences in skeletal size, however, disappeared over the nestling period. Additionally, there was no variation in plasma T levels among treatment groups by the middle of the nestling period.

At 2 wk posthatch, high-dose chicks were significantly heavier, more mature, and in better condition than chicks in the other two treatment groups. In contrast, high doses of T had a clearly inhibitory effect on cell-mediated immunity.

Our observation that high-dose nestlings were significantly heavier, more mature, and in better condition 2 wk after hatching is consistent with results found by Schwabl (1996) in which high-yolk androgen levels resulted in canary chicks that begged more and grew faster. We did not quantify begging behavior in these nestlings, but it is possible that offspring in the high-dose group begged more, received more food, and thus gained more weight than offspring in the other two groups.

Because we did not see differences in plasma T levels among treatment groups on day 10 posthatch, it is likely that the weight differences that we saw were caused by organizational effects on the embryo caused by high levels of yolk T. In other words, early exposure to T might permanently change the way an individual physiologically and behaviorally responds, ultimately affecting processes associated with weight gain. For example, in rats, treatment with sex steroids during the neonatal period has been found to be responsible for permanently altering patterns of glucocorticoid secretion (McCormick et al. 1998) as well as "imprinting" sexually dimorphic patterns of growth hormone release (Jansson et al. 1985; Painson et al. 2000). Finally, early exposure to T has been shown to alter sex steroid receptor concentrations permanently in the rat (Kuhnemann et al. 1995) as well as testosterone-metabolizing enzymes in the zebra finch (Vockel et al. 1990), altering the way an individual responds to hormonal stimuli during adulthood. Although most of these effects have been demonstrated in mammals and have yet to be examined in birds, the major axes that control the release of growth hormone, glucocorticoids, and sex steroids are highly conserved and likely to be similar in birds and mammals. As a result, yolk T can have permanent effects on processes associated with weight gain, such as lipid mobilization, muscle breakdown, and behavioral responses through adulthood, which may explain the body mass differences we saw among treatment groups.

Our observation that yolk T had a suppressive effect on the T-cell immune responses in these nestlings is consistent with the findings of many studies examining the effects of androgens on immunity. Androgen receptors have been found in the thymus (the site of T-cell maturation and differentiation) in den-

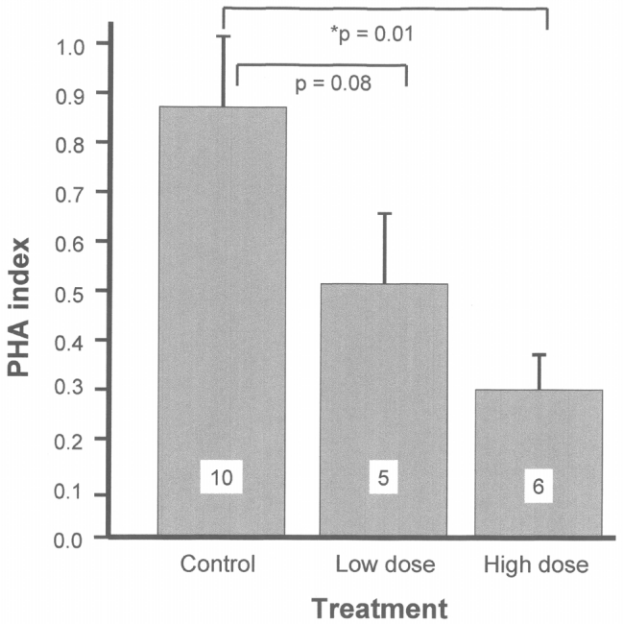

Figure 6. The effects of in ovo testosterone and control injection treatments on mean (±SE) phytohemagglutinin (*PHA*) index (calculated as described in Fair and Meyers [2002]). Injection treatments are the same as those described in Figure 1.

sities comparable to those found in the reproductive tract (Viselli et al. 1995), and the stimulation of these androgen receptors causes a shift in the thymic cell population toward apoptosis, while castration shifts the population toward maturation as helper or cytotoxic T-cells (Olsen et al. 1991; Olsen and Kovacs 1996). It has been suggested that, overall, androgens generally suppress cytotoxic/suppressor T-cell function, but the effects seen in the peripheral immune system are most likely caused by alterations within the thymocyte population (Olsen and Kovacs 1996). Although numbers of circulating leukocytes did not differ among treatment groups in this study, chicks exposed to high levels of T during the embryonic period may have developed an immune system with a smaller number of mature, functional T-cells. Additionally, T has been shown to induce directly oxidative stress in many tissues (von Schantz et al. 1999), which damages lymphocytes involved in the immune response and results in immunosuppression (Raberg et al. 1998). This phenomenon could explain the decreased swelling responses of high-T nestlings.

Because previous experiments show that T has an immunosuppressive effect on the development of humoral immunity (Glick 1961; Norton and Wira 1977; Deyhim et al. 1992), we also expected chicks in the high-dose group to exhibit lower antibody titers than chicks in the other two groups. However, the number of nestlings that responded to our SRBC challenge was minimal. Because we were able to wait only 10 d after the initial antigen injection (because of the length of the nestling period), the time period may not have been long enough for us to witness the production of antibodies to the SRBCs. In other avian studies, researchers have waited 2 wk after SRBC injection before testing for antibodies to SRBCs (Smits and Williams 1999). Therefore, our results concerning the humoral immune response are inconclusive.

We attempted to encompass several aspects of the immune system in this study in an attempt to assess overall immunocompetence. Although our PHA challenge detected treatment differences in T lymphocyte responsiveness, it will be important in future studies to assess humoral immune function. Additionally, a good general assessment of the overall immune response would be a challenge with a novel antigen normally encountered in the environment, and we encourage the inclusion of such a test in future studies.

Our results suggest that yolk androgens have a variety of flexible context-dependent effects on the development and survival of avian nestlings. When breeding density is great and competition is high, offspring may benefit from high levels of yolk T because they will be able to beg more and/or gain weight more efficiently during the nestling period. The immunosuppressive effects of high yolk T, however, leave the resulting offspring vulnerable to infection by pathogens, so the likelihood of parasitism will trade off against improved growth and development. In this way, environmental and social conditions mediate the fitness effects of the allocation of yolk androgens.

Testosterone acts as a signal used to control and coordinate the allocation of resources to different processes within the body. The alteration of reproductive success through the allocation of yolk T is an extremely complicated phenomenon that involves the integration of environmental, social, and physiological effects. The optimal level of yolk T will most likely vary between male and female offspring as well as among offspring at different positions within the clutch order and offspring reared in different environments. Therefore, we must think of the adaptive significance of differential yolk T allocation in terms of a range of adaptive responses to a range of environmental challenges.

Acknowledgments

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Literature Cited

Casto J.M., V. Nolan Jr., and E. Ketterson. 2001. Steroid hormones and immune function: experimental studies in wild and captive dark-eyed juncos. Am Nat 157:408–420.

Corvol M., O. Blanchard, and L. Tsagris. 1992. Bone and cartilage responsiveness to sex steroids. J Steroid Biochem Mol Biol 43:415–418.

Dein F.J. 1986. Chemical avian medicine and surgery. Pp. 174–191 in L.A. Harrison, ed. Hematology. Saunders, Philadelphia.

Deyhim F., R.E. Moreng, and E.W. Kienholz. 1992. The effects of testosterone propionate on growth of broiler chicks. Poult Sci 71:1921–1926.

Duckworth R.A., M.T. Mendonça, and G.E. Hill. 2001. A condition dependent link between testosterone and disease resistance in the house finch. Proc R Soc Lond B Biol Sci 268: 2467–2472.

Fair J.M. and O.B. Myers. 2002. The ecological and physiological costs of lead shot and immunological challenge to developing Western bluebirds. Ecotoxicology 11:199–208.

Fischer L.M., D. Catz, and D.B. Kelley. 1995. Androgen-directed development of the *Xenopus laevis* larynx: control of androgen receptor expression and tissue differentiation. Dev Biol 170:115–126.

French J.B., Jr., I.C.T. Nisbet, and H. Schwabl. 2001. Maternal steroids and contaminants in common tern eggs: a mechanism of endocrine disruption? Comp Biochem Physiol C 128: 91–98.

Gil D., J. Graves, N. Hazon, and A. Wells. 1999. Male attrac-

tiveness and differential testosterone investment in zebra finch eggs. Science 286:126–128.

Glick B. 1961. The influence of dipping eggs in male hormone solutions on lymphatic tissue and antibody response of chickens. Endocrinology 69:984.

Goto N., H. Kodama, K. Okada, and Y. Fujimoto. 1978. Suppression of phytohaemagglutinin skin response in thymectomized chickens. Poult Sci 57:246–250.

Groothuis T.G. and H. Schwabl. 2002. Determinants of within- and among-clutch variation in levels of maternal hormones in black-headed gull eggs. Funct Ecol 16:281–289.

Hario M. 2001. Chick growth and nest departure in Baltic black guillemots *Cepphus grylle*. Ornis Fenn 78:97–108.

Hay L. and F.C. Hudson. 1989. Practical Immunology. Blackwell, Oxford.

Henry M.H. and W.H. Burke. 1999. The effects of in ovo administration of testosterone or an antiandrogen on growth of chick embryos and embryonic muscle characteristics. Poult Sci 78:1006–1013.

Jansson J.-O., S. Ekberg, O. Isaksson, A. Mode, and J.-A. Gustafson. 1985. Imprinting of growth hormone secretion, body growth, and hepatic steroid metabolism by neonatal testosterone. Endocrinology 117:1881–1889.

Janzen F.J., M.E. Wilson, J.K. Tucker, and S.P. Ford. 1998. Endogenous yolk steroid hormones in turtles with different sex-determining mechanisms. Gen Comp Endocrinol 111:306–317.

Kasperk C., R. Fitzsimmons, D. Strong, S. Mohan, J. Jennings, J. Werdegal, and D. Baylink. 1990. Studies of the mechanisms by which androgens enhance mitogenesis and differentiation in bone cells. J Clin Endocrinol Metab 71:1322–1329.

Ketterson E. and V. Nolan. 1999. Adaptation, exaptation, and contraint: a hormonal perspective. Am Nat 154(suppl.):S4–S25.

Ketterson E.D., V. Nolan Jr., L. Wolf, and C. Ziegenfus. 1992. Testosterone and avian life histories: effects of experimentally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco. Am Nat 140:980–999.

Kuhnemann S., T.J. Brown, R.B. Hochberg, and N.J. MacLusky. 1995. Sexual differentiation of estrogen receptor concentrations in the rat brain: effects of neonatal testosterone exposure. Brain Res 691:229–234.

Ling S., A. Dai, M.R.I. Williams, K. Myles, R.J. Dilley, P.A. Komesaroff, and K. Sudhir. 2002. Testosterone enhances apoptosis-related damage in human vascular endothelial cells. Endocrinology 143:1119–1125.

Lipar J.L. and E.D. Ketterson. 2000. Maternally derived yolk testosterone enhances the development of the hatching muscle in red-winged blackbird *Agelaius phoeniceus*. Proc R Soc Lond B Biol Sci 267:2005–2010.

Lipar J.L., E.D. Ketterson, and V. Nolan Jr. 1995. Steroid hormones in the yolk of red-winged blackbird eggs. Poult Avian Biol Rev 6:329.

Lochmiller R.L., M.R. Vestey, and J.C. Boren. 1993. Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. Auk 110:503–510.

Lovern M.B. and J. Wade. 2001. Maternal plasma and egg yolk testosterone concentrations during embryonic development in green anoles (*Anolis carolinensis*). Gen Comp Endocrinol 124:226–235.

McCormick C.M., B.F. Furey, M. Child, M.J. Sawyer, and S.M. Donohue. 1998. Neonatal sex hormones have "organizational" effects on the hypothalamic-pituitary-adrenal axis of male rats. Dev Brain Res 105:295–307.

Mendonça M.T., S.D. Chernetsky, K.E. Nester, and G.L. Gardner. 1996. Effects on gonadal sex steroids on sexual behavior in the big brown bat *Eptesicus fuscus*, upon arousal from hibernation. Horm Behav 30:153–161.

Mu X. and G.A. LeBlanc. 2002. Developmental toxicity of testosterone in the crustacean *Daphnia magna* involves anti-ecdysteroidal activity. Gen Comp Endocrinol 129:127–133.

Nolan V., Jr., E. Ketterson, C. Ziegenfus, D.P. Cullen, and C.R. Chandler. 1992. Testosterone and avian life histories: effects of experimentally elevated testosterone on prebasic molt and survival in male dark-eyed juncos. Condor 94:364–370.

Norton J.M. and C.R. Wira. 1977. Dose-related effects of the sex hormones and cortisol on the growth of the bursa of Fabricius in chick embryos. Steroid Biochem 122:2617.

Olsen N.J. and W.J. Kovacs. 1996. Gonadal steroids and immunity. Endocr Rev 17:369–384.

Olsen N.J., M.B. Watson, G.S. Henderson, and W.J. Kovacs. 1991. Androgen deprivation induces phenotypic and functional changes in the thymus of adult male mice. Endocrinology 129:2471–2476.

Painson J., J.D. Veldhuis, and G. Tannenbaum. 2000. Single exposure to testosterone in adulthood rapidly induces regularity in the growth hormone release process. Am J Physiol 278:E933–E940.

Raberg L., M. Grahn, D. Hasselquist, and E. Svensson. 1998. On the adaptive significance of stress-induced immunosuppression. Proc R Soc Lond B Biol Sci 265:1637–1641.

Reed W. and C.M. Vleck. 2001. Functional significance of variation in egg-yolk androgens in the American coot. Oecologia 128:164–171.

Richner H., A. Oppliger, and C. Philippe. 1993. Effect of an ectoparasite on reproduction in great tits. J Anim Ecol 62: 703–710.

Royle N.J., P.F. Surai, and I.R. Hartley. 2001. Maternally derived androgens and antioxidants in bird eggs: complementary but opposing effects? Behav Ecol 12:381–385.

Schwabl H. 1993. Yolk is a source of maternal testosterone for developing birds. Proc Natl Acad Sci USA 90:11446–11450.

———. 1996. Maternal testosterone in the avian egg enhances postnatal growth. Comp Biochem Physiol A 114:271–276.

———. 1997*a*. The contents of maternal testosterone in house

sparrow *Passer domesticus* vary with breeding conditions. Naturwissenschaften 84:406–408.

———. 1997*b*. Control of offspring fitness by maternal hormones. Pp. 1677–1682 in S. Kikuyama, ed. Advances in Comparative Endocrinology. Monduzzi Editore, Bologna.

Schwabl H. and J.L. Lipar. 2002. Hormonal regulation of begging behaviour. Pp. 221–244 in M. Leonard, ed. The Evolution of Nestling Begging: Competition, Cooperation, and Communication. Kluwer Academic, Dordrecht.

Schwabl H., D. Mock, and J. Gieg. 1997. A hormonal mechanism for parental favouritism. Nature 386:231.

Smits J.E. and T.D. Williams. 1999. Validation of immunotoxicology techniques in passerine chicks exposed to oil sands tailings water. Ecotoxicol Environ Saf 44:105–112.

Sockman K.W. and H. Schwabl. 2000. Yolk androgens reduce offspring survival. Proc R Soc Lond B Biol Sci 267:1451–1456.

Sockman K.W., H. Schwabl, and P.J. Sharp. 2001. Regulation of yolk-androgen concentrations by plasma prolactin in the American kestrel. Horm Behav 40:462–471.

Viselli S.M., N.J. Olsen, K. Shults, G. Steizer, and W.J. Kovacs. 1995. Immunochemical and flow cytometric analysis of androgen receptor expression in thymocytes. Mol Cell Endocrinol 109:19–26.

Vockel A., E. Prove, and J. Balthazart. 1990. Effects of castration and testosterone treatment on the activity of testosterone-metabolizing enzymes in the brain of male and female zebra finches. J Neurobiol 21:808–825.

von Schantz T., S. Bensch, M. Grahm, D. Hasselquist, and A. Wittzell. 1999. Good genes, oxidative stress, and condition-dependent sexual signals. Proc R Soc Lond B Biol Sci 266: 1–12.

Whittingham L.A. and H. Schwabl. 2002. Maternal testosterone in tree swallow eggs varies with female aggression. Anim Behav 63:63–67.

Wingfield J.C., S. Lynn, and K. Soma. 2001. Avoiding the "costs" of testosterone: ecological basis of hormone-behavior interactions. Brain Behav Evol 57:239–251.

Winkler D.W. 1993. Testosterone in egg yolks: an ornithologist's perspective. Proc Natl Acad Sci USA 90:11439–11441.

Yom-Tov Y. 2001. Global warming and body mass decline in Israeli passerine birds. Proc R Soc Lond B Biol Sci 268:947–952.