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Individual variation in plasma estradiol-17β and androgen levels during egg formation in the European starling *Sturnus vulgaris*: implications for regulation of yolk steroids

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Abstract

While it is clear that maternal transfer of steroids to egg yolk can have significant effects on offspring phenotype, an unresolved question is whether females can facultatively adjust yolk hormone levels independently of their own plasma levels or whether yolk steroid levels are simply a direct consequence of temporal variation in the female's hormonal status. In part, this is because we lack detailed information about the day-to-day pattern of changes in plasma hormone levels during the laying cycle for non-domesticated birds. Here, we describe changes in plasma estradiol-17β (E2) and androgens, throughout laying in relation to specific stages of ovarian follicular development in the European starling (*Sturnus vulgaris*). Plasma E2 levels increased rapidly from the onset of rapid yolk development (RYD) to reach maximum levels in birds with a complete follicle hierarchy ($\geqslant 4$ yolky follicles). However, levels decreased linearly throughout the later stages of follicle development returning to pre-breeding values *before* the final yolky follicle was ovulated. In females with $\geqslant 4$ yolky follicles there was 10-fold variation in plasma E2 levels among individual females, but this was not related to plasma levels of the main yolk precursor vitellogenin or to the total mass of yolky follicles developing at the time of blood sampling. In contrast to E2, plasma androgen levels showed only a very gradual linear decline throughout the laying cycle from pre-RYD to clutch completion. Furthermore, androgen levels showed less individual variability: 4-fold variation among females with $\geqslant 4$ yolky follicles, although this was also independent of our measures of reproductive function. Data on inter- and intra-individual variation in female hormone levels are important to set-up a priori predictions for, and interpretation of, studies of yolk hormone levels.

Keywords: *Sturnus vulgaris*; Estradiol-17β; Androgens; Egg formation; Maternal effects

1. Introduction

Many recent studies have shown that maternal transfer of steroids to egg yolk can have significant effects on various aspects of offspring phenotype in oviparous vertebrates including embryo development, early post-hatching growth, begging behaviour, and subsequent social status (e.g., Eising et al., 2001; Lipar and Ketterson, 2000; Schwabl, 1996a; but see Sockman and Schwabl, 2000). Systematic variation in yolk steroid hormone levels has been reported within-clutches, in relation to laying order, in many species, e.g., in some species androgen levels increase over the laying sequence (French et al., 2001; Lipar et al., 1999; Schwabl, 1993), but in others hormone levels decrease or do not change (Gil et al., 1999; Schwabl et al., 1997; Whittingham and Schwabl, 2002). However, there is also marked variation in yolk steroid levels among clutches laid by different females, and individual variation in yolk androgen levels has been shown to be related to a female's level of social stimulation, aggressiveness, or social status (Groothuis and Schwabl, 2002; Muller et al., 2002; Whittingham and Schwabl, 2002), or to other environmental conditions the female experiences during egg formation (Bowden et al., 2002; Schwabl, 1996b).

It has been suggested that yolk hormone levels correlate with circulating plasma hormone levels in the female

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(Schwabl, 1996b; but see Hackl et al., 2003 and Discussion). Furthermore, several studies have shown that experimental elevation of maternal hormone levels results in a corresponding increase in hormone levels in eggs laid by these females (Adkins-Regan et al., 1995; Janzen et al., 2002). However, a major unresolved question is whether females can facultatively adjust yolk hormone levels in a sequence-specific manner through laying, independently of their own plasma levels, or whether the observed variation in yolk steroids is simply a direct consequence of temporal variation in the female's hormonal status (Birkhead et al., 2000). If females themselves have specific time- or laying-sequence dependent requirements for changes in plasma hormone levels dictated by the endocrine control mechanisms underlying reproductive function or egg formation (e.g., for estrogens or progestins, Johnson, 2000) then this might determine, or even constrain, patterns of yolk hormones if these simply reflect the mother's hormonal milieu.

The resolution of this problem has been hindered by a lack of detailed information about the day-to-day pattern of changes in plasma hormone levels during the laying cycle. Although there have been many studies on the reproductive endocrinology of breeding birds (e.g., Dawson, 1983) these only report changes in plasma hormone levels for broad reproductive stages (''non-breeding,'' ''laying,'' or ''incubating,'' etc). Other than for domesticated poultry (Etches, 1996), very little is known about the pattern of variation in gonadal steroids through the cycle of follicular development, ovulation and oviposition. Yet these data are very important because they provide the hormonal ''background'' against which variation in yolk steroids must be interpreted. Here, we describe changes in two of the major gonadal steroids, estradiol-17β and androgens, throughout laying in relation to specific stages of ovarian follicular development in the European starling (*Sturnus vulgaris*). Experimental manipulation of plasma estradiol levels has been shown to have marked effects on offspring phenotype, survival and secondary sex ratio (Adkins-Regan et al., 1995; Williams, 1999). We also describe individual variation in estradiol-17β levels, controlling for variation in reproductive state, and relate this variation to variation in other estrogen-dependent traits (plasma yolk precursor levels, follicle mass, etc). Finally, we consider the implications of these data for interpretation of the mechanisms underlying variation in yolk steroids.

2. Materials and methods

2.1. Fieldwork, blood sampling, and collection

Fieldwork was carried out at the Pacific Agri-Food Research Center (PARC) in Agassiz BC, Canada (49°14′N, 121°46′W) between April and June 2001, under a Simon Fraser University animal care permit (499B), following guidelines of the Canadian Council on Animal Care. The site consists of c. 125 nest-boxes on farm buildings and telephone poles that are used each year by breeding starlings. All boxes were checked daily to determine dates of clutch initiation and clutch completion and the laying sequence of eggs. During laying and early chick-rearing females were taken from their nest boxes during night-time (generally between 20:00 and 24:00 h); during late chick-rearing provisioning females were trap-caught (always within an hour before sun set). Non-breeding and pre-laying females were mist-netted at two barns at the same site that were used as roosting sites. Eggs were collected when present at the time females were collected, for mass and size measurements; however, these were used for another experiment so data on yolk steroid concentrations were not obtained in the present study.

Prior to laying of the first egg the reproductive status of individual females was unknown so random-caught birds were collected during the 10 days before the first egg appeared in the colony (pre-laying birds, $n = 25$). These birds were therefore at different stages of ovarian follicle development and oviduct growth, but included no females that had laid their first egg. After recording the first clutch initiation in the colony we started collecting females at all stages of egg laying (eggs 1–6) through to clutch completion (total $n = 55$). In addition, birds were collected at the end of the wintering period (pre-breeding, $n = 17$), at the one-egg stage of laying ($n = 14$), and during chick provisioning ($n = 19$).

All birds were blood sampled from the jugular vein by exsanguination under anesthesia (ketamine:xylasine at doses of 20 and 4 mg/kg, respectively) 4–5 h after capture (during this time RMR was measured on all birds, see Vézina and Williams, 2002). Blood was centrifuged immediately at 5000 rpm for 10 min and plasma was stored at −20 °C until assayed. We confirmed that time of blood sampling after capture did not affect plasma E2 levels by comparing levels in 1-egg birds in 2001 ($n = 13$) to a sample of 1-egg birds assayed in 1997 ($n = 6$) that were blood sampled immediately after capture (T.D. Williams, unpublised data). Mean plasma E2 concentrations did not differ for these two sampling methods (1997, 272 ± 43 vs. 2001, 209 ± 33 pg/ml; $t_{17} = 1.10$, $p = 0.28$). Birds were dissected to records the number of follicles and the presence of post-ovulatory follicles allowing us to confirm the precise stage of ovarian development for every bird. We recorded the fresh mass of the follicle-free ovary and the individual weights of all ovarian follicles (±0.001 g), but these were used for another study and were not available for measurement of yolk hormones in this study.

2.2. Hormone and vitellogenin assays

Hormone concentrations were measured with radioimmunoassay (RIA) in duplicate for each sample after extraction in dichloromethane (for a detailed description of the analysis see Wingfield and Farner, 1975). Plasma volumes of 300 and 100 μl were used for measurement of estradiol-17β and androgens, respectively. A small amount of tritiated steroid label (2000 cpm) was added to each sample prior to dichloromethane extraction to control for loss of the steroid during extraction. Recoveries (E2 = 60.8% ± 0.95 SE, and androgens = 62.1% ± 0.81 SE) of tritiated steroids after extraction were used to adjust assayed concentrations of steroids. The antibodies used to examine steroid concentrations were: estradiol-17β antibody 1702 (Arnel, New York, NY) and testosterone antibody 01916 A-Z (Wien, Succasunna, NJ). The estradiol antiserum used in this study is highly specific for estradiol-17β and its cross-reactivities with other steroids are negligible. The testosterone antiserum used in this study cross-reacts with testosterone by 100%, 5-α-dihydrotestosterone (DHT) by ~60%, and with 5-β-DHT by about 6%. Inter- and intra-assay coefficients of variation were 2 and 4%, and 3 and 5%, for E2, and T, respectively. The sensitivity of the RIA(s) was 1.95 pg/tube for both E2 and T.

Plasma vitellogenin (VTG) was assayed indirectly using the zinc method developed for the domestic hen (Mitchell and Carlisle, 1991) which we have validated for passerines (e.g. Williams and Martyniuk, 1999) including the European starling (Challenger et al., 2001; Vézina and Williams, 2003). This method measures total plasma zinc, and then separates the zinc bound to serum albumen from that bound to VTG and very-low density lipoprotein (VLDL) by depletion of VTG and VLDL from the plasma sample by precipitation with dextran sulfate. The depleted plasma sample is then assayed for zinc and vitellogenic zinc (VTG-Zn) is calculated as the difference between total and depleted zinc; VLDL accounts for only 2% of total plasma zinc (Mitchell and Carlisle, 1991). The concentration of VTG-Zn is proportional to the plasma concentration of plasma VTG (Mitchell and Carlisle, 1991). Intra- and inter-assay variation (CV%) for VTG determined using a laying hen plasma pool were 7.2 and 16.3%, respectively. All assays were run using 96-well microplates, and measured using a Biotek 340i microplate reader.

2.3. Statistical analysis

All statistical analyses were carried out using SAS (SAS Institute, 1989). Variation in hormone levels with stage of ovarian development were analysed using ANOVA with stage as a factor and Bonferroni adjustment of significance level for post-hoc multiple comparison tests (proc GLM). The relationship between hormone levels and other reproductive traits was analysed using Pearson correlation. Values are presented as means ± SE unless otherwise stated.

3. Results

Plasma E2 levels varied significantly with stage of ovarian development ($F_{9,107} = 8.86$, $p < 0.001$; Fig. 1), increasing from low levels (55 pg/ml) in non-breeders to 201 pg/ml in pre-laying birds with 3–4 yolky follicles ($p = 0.030$). E2 levels then remained high (>170 pg/ml) in pre-laying birds with 5–6 yolky follicles and laying birds with ⩾2 yolky follicles ($p = 1.0$), before decreasing rapidly to 53 pg/ml in laying birds with no yolky follicle remaining but with an oviductal egg ($p = 0.026$). In laying females with a full follicle hierarchy of 4–5 follicles, plasma E2 varied 10-fold from 44–423 pg/ml. As we have previously reported (e.g., Challenger et al., 2001; Vézina and Williams, 2003) plasma VTG also varied significantly with stage of ovarian development ($F_{8,81} = 16.21$, $p < 0.0001$). However, although plasma VTG and E2 increased in parallel with onset of follicle development in pre-laying birds, E2 decreased approximately 48 h earlier than the decrease in VTG at the end of laying (see Fig. 1).

Plasma androgen levels also varied with stage of ovarian development ($F_{9,107} = 2.95$, $p = 0.004$; Fig. 1), but levels were much less variable between stages, and only one of the pair-wise comparisons was significant (pre-laying birds with no follicles vs. clutch completion

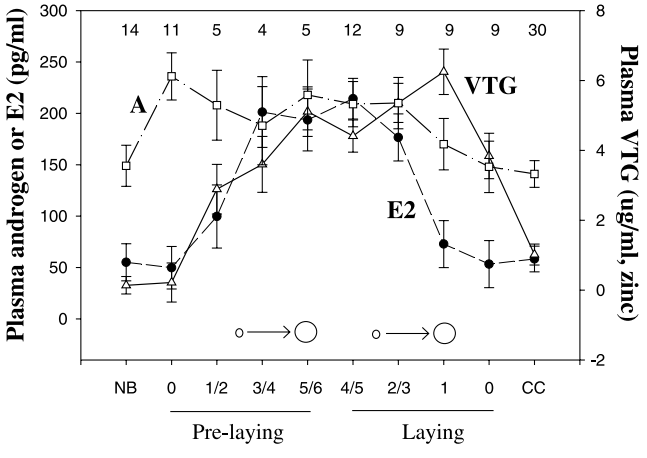

Fig. 1. Variation in plasma estradiol-17β (E2, closed circles), androgen (A, open squares) and vitellogenin (VTG, open triangles) levels during the laying cycle, in relation to the number of developing, yolky follicles present in the ovary for the pre-laying and laying phase. NB, non-breeder; CC, clutch completion; 0, zero; 1/2, one or two follicles; etc. Circles and arrows indicate timing of follicle development for an early- and late-developing follicle (interval between two follicle stages = c. 48 h). Values are means ± SE, with sample sizes for each stage given at the top of the graph.

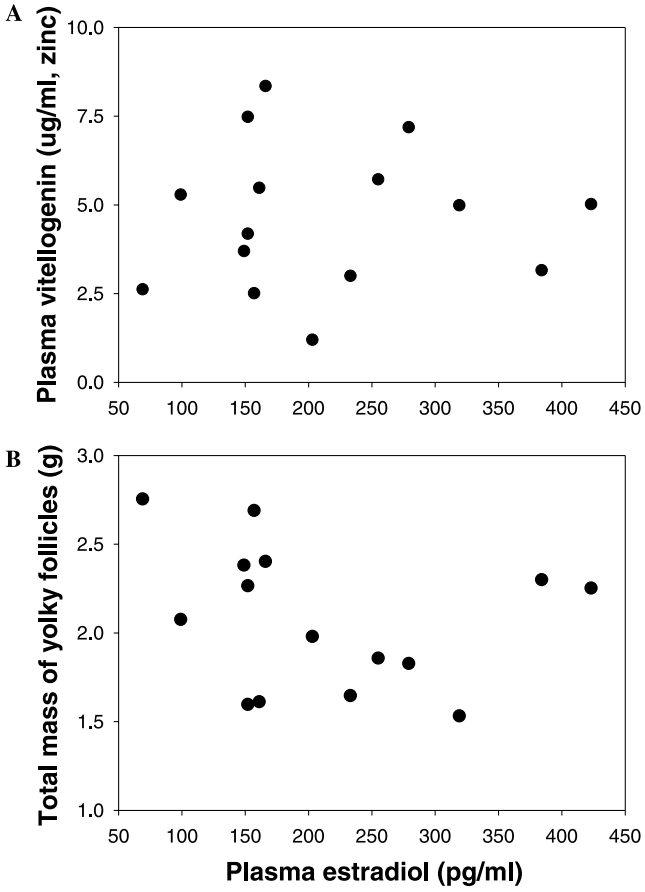

Fig. 2. Relationship between plasma estradiol-17β levels and (A) plasma vitellogenin levels (top), and (B) total mass of yolky follicles (bottom), in females with $\geqslant 4$ yolky follicles.

birds, $p = 0.011$). In laying females with a full follicle hierarchy of 4–5 follicles, plasma androgen levels varied 4-fold from 93–383 pg/ml.

In females with $\geqslant 4$ yolky follicles both plasma VTG levels ($r_{16} = 0.06$, $p = 0.84$; Fig. 2A) and the total mass of yolky follicles ($r_{16} = -0.26$, $p = 0.34$; Fig. 2B) were independent of plasma E2 levels (note: one outlier was excluded from these analyses: a bird with total follicle mass = 0.59 g and F1 follicle mass = 0.387 g, cf. range of data in Fig. 2; this bird had a plasma E2 level of 295 pg/ml). Similarly, both of these reproductive parameters were independent of plasma androgen levels in these birds (VTG, $r_{16} = -0.34$, $p = 0.19$; follicle mass, $r_{16} = -0.31$, $p = 0.24$). Finally, in females with $\geqslant 4$ yolky follicles there was no correlation between plasma E2 and plasma androgen levels within individuals ($r_{16} = 0.42$, $p = 0.19$).

4. Discussion

In European starlings, plasma levels of estradiol-17β increased rapidly from the onset of rapid yolk development (RYD) to reach maximum levels in birds with a complete follicle hierarchy ($\geqslant 4$ yolky follicles). However, levels decreased linearly throughout the later stages of follicle development returning to pre-breeding values *before* the final yolky follicle was ovulated. In females with $\geqslant 4$ yolky follicles there was 10-fold variation in plasma E2 levels among individual females, but this was not related to plasma levels of the main yolk precursor vitellogenin or to the total mass of yolky follicles developing at the time of blood sampling. In contrast to E2, plasma androgen levels showed only a very gradual linear decline throughout the laying cycle from pre-RYD to clutch completion. Furthermore, androgen levels showed less individual variability: 4-fold variation among females with $\geqslant 4$ yolky follicles.

4.1. Variation in hormone levels and female reproductive function

There have been few other studies describing detailed changes in hormone levels on a day-to-day basis during the laying cycle for non-poultry avian species. Sockman and Schwabl (2001) measured fecal estradiol-17β in canaries (*Serinus canaria*) during the laying cycle and reported a similar pattern to the one seen for plasma E2 in our study in starlings. In female canaries fecal E2 increased sharply to a peak at the 1-egg stage and then decreased linearly, reaching "low levels" three days after the onset of laying when the fourth (last) egg was laid. Sockman and Schwabl (2001) further showed that the decrease in fecal E2 levels was closely associated with onset of incubation. Although in our study we did not have information on timing of onset of incubation, hatching in our starling colony is highly synchronous (within a 24 h period) suggesting that birds start incubation with the laying of the penultimate, or last egg (as reported by Meijer, 1990). Thus, in European starlings the decrease in plasma E2 between birds with 2–3 yolky follicles remaining to be ovulated and those with only one yolky follicle remaining would also be coincident with onset of incubation.

It is well known that estrogens play a fundamental role in regulating female reproduction, including yolk precursor production (Wallace, 1985; Walzem et al., 1999), oviduct development (Brant and Nalbandov, 1956; Yu et al., 1971), and reproductive behaviour (e.g., Balthazart, 1983). Furthermore, production of E2 is highest in small, early stage follicles (Bahr et al., 1983) such that total ovarian output of E2 would be predicted to peak at the onset of ovulation (Sockman and Schwabl, 2001, this study). Thus, the rapid increase in E2 prior to onset of laying is not surprising and is consistent with the known physiological functions of this hormone. However, in starlings, plasma E2 levels decrease after onset of laying well before there is any detectable decrease in the other "estrogen-dependent"

components of the reproductive system. For example, plasma E2 levels decreased approximately 48 h before any decline in plasma VTG levels (see Fig. 2). Thus, high circulating levels of yolk precursors can be maintained until after the last yolky follicle has ovulated (see also Challenger et al., 2001) without maintenance of high plasma E2 levels. The most parsimonious explanation for this is that the relatively long half-life of VTG (1–2 days; Redshaw and Follett, 1976; Schultz et al., 2001) coupled with high circulating levels earlier in RYD, allow maintenance of these levels even if there is a decrease in E2-stimulated VTG production. Similarly, oviduct size and function is maintained until the last follicle is ovulated and the oviduct then starts to regress rapidly while still retaining the last oviductal egg (starlings, Vézina and Williams, 2003; zebra finches, Williams and Ames, 2004). Although oviduct growth is clearly E2-dependent (see above) this again suggests that subsequent oviduct function can be maintained in the absence of high circulating E2 levels. It therefore appears that plasma E2 levels are elevated in laying females for a very short period; why does E2 decrease rapidly even before females have completed the egg production and laying process? One reason for this might be that there are negative, non-reproductive, effects of high estrogen levels which would select for rapid down-regulation, e.g., suppression of hematopoiesis (Clermont and Schrar, 1979) or immunosuppression (al-Afaleq and Homeida, 1998 , but see Leitner et al., 1996), or decreased embryo viability, especially of male embryos (Williams, 1999). Alternatively, Monroe et al. (2000) showed that estrogens oppose the induction of apoptosis that is involved in oviduct regression, perhaps suggesting that the decrease in plasma E2 is actually a pre-requisite for rapid oviduct regression at the end of laying (Williams and Ames, 2004).

In females at similar stages of ovarian development (with $\geqslant 4$ yolky follicles) there was 10-fold variation in plasma E2 levels among individuals. However, this variation was not explained by variation in E2-dependent yolk precursor levels (which also varied 10-fold) or the total mass of developing, yolky follicles. We did not have information on clutch size for most of the birds in our study, but Sockman and Schwabl (2001) found that clutch size did not explain variation in fecal E2 levels in canaries during the laying cycle. These data are consistent with other studies that have reported large inter-individual variation in different physiological or reproductive traits in laying birds, with little or no systematic relationship between traits (even though there is often good evidence that these traits are mechanistically linked; Christians and Williams, 2001; Salvante and Williams, 2002). Within the cost-benefit framework discussed above, this seemingly ''random'' large-scale variation presents a paradox: if there are costs of high hormone levels, selection should generate a match between physiological capacity (hormone level) and functional demand (the amount of hormone required; sensu Diamond and Hammond, 1992). A possible explanation for this paradox is that individuals have very different sensitivities to circulating E2 levels, such that in different individuals very different plasma E2 levels are required to support the same level of physiological function. In some situations individual variation in yolk hormone levels can be related to variation in timing components of reproductive quality (Bowden et al., 2002) or variation in reproductive behaviour (Pilz et al., 2003). Nevertheless, it is clear that more attention needs to be focused on individual variation in plasma hormone profiles in this context.

Since the antibody used in our assay cross-reacted with testosterone and 5-α-dihydrotestosterone (DHT) we are not able to dissociate the pattern of variation for these two hormones. However, several studies have shown that plasma T and DHT levels are positively correlated, at least in males ($r \geqslant 0.7$; Hau et al., 2000; Wikelski et al., 2000). Very little is known about the role of, or requirements for, androgens during egg formation in female birds (in marked contrast to the number of studies on yolk androgens), although androgens induce protein synthesis in the oviduct of E2-primed birds (Johnson, 2000). Schwabl (1996a) measured fecal testosterone in female canaries and showed that fecal levels increased before the first egg was laid and then decreased through laying of subsequent eggs, i.e., this species expressed a different pattern than we report (with the exact timing of the decrease being dependent on photoperiod and the onset of incubation). Intraspecific female aggression is relatively common in breeding starlings (Sandell and Smith, 1997) and females are most aggressive during the pre-laying period and less so during egg-laying. The gradual decrease in plasma androgens that we report might therefore be related to the seasonal decline in female aggressive behaviours.

4.2. Implications for variation in yolk steroid levels

There are two key results from our study which have important implications for predicting variation in yolk hormone levels. First, the magnitude of the temporal change in plasma E2 levels (4-fold variation) was much greater than that for plasma androgens (none) through the cycle of ovarian development. Secondly, in birds at the same reproductive stage inter-individual variation in plasma E2 (>10-fold) was much greater than variation in plasma androgens (3-fold). Whether yolk hormone levels are a simple, direct reflection of maternal hormone levels, or whether females can adjust yolk hormone levels independently of their own plasma levels remains an unresolved question (Birkhead et al., 2000). Hackl et al. (2003) recently suggested that >99% of yolk steroids come directly from the cells of the follicular wall

rather than from the peripheral circulation. However, their study used a single injection of radiolabelled androgen, most of which was removed very rapidly from the circulation: very different from the chronic, sustained pattern of endogenous hormone levels. As Hackl et al. (2003) pointed out (p. 330) they found no difference in yolk hormones with laying sequence which contrasts with the hormonal situation described for other species. Hackl et al.'s (2003) results do not explain the correlation between maternal fecal, plasma and yolk steroid levels (e.g., Schwabl, 1996b), why treatment of laying female quail with estradiol benzoate results in elevated yolk estradiol (Adkins-Regan et al., 1995), or why factors which elevate plasma steroid levels (e.g., social factors) also elevate yolk levels. If yolk steroid levels do simply reflect the mother's plasma levels, at least in some species, our data would clearly predict that in the European starling inter- and intra-clutch variation in yolk E2 would be much greater than for yolk androgens. Furthermore, since maternal E2 levels are very different for early- vs. late-developing yolks (see Fig. 1), if yolk steroids simply reflect the mother's plasma levels, then our data predict that first-laid eggs will have much higher yolk E2 than later-laid eggs, but that there will be no difference for yolk androgens. Pilz et al. (2003) reported that yolk T levels increased with laying order in this species, perhaps providing support for the suggestion that female birds can manipulate yolk hormone levels independently of their own plasma levels. This highlights the value of data on female hormone levels to set up a priori predictions for, and interpretation of, studies of yolk hormone levels (cf. most studies to date).

Finally, the large-scale inter-individual variation in plasma hormone levels we report (see also Groothuis and Schwabl, 2002; Schwabl, 1996b) presents a further paradox with regard to the significance of intra-individual variation in yolk steroid levels. In most species where laying-sequence specific variation in yolk steroids have been reported, the average intra-clutch difference (e.g., between first- and last-laid egg) is generally quite small. For example, for yolk testosterone this differences is typically two-fold or less (e.g., Gil et al., 1999; Pilz et al., 2003; Schwabl, 1996b), i.e., less than the magnitude of inter-individual variation in plasma levels that we report. Again, this might provide support for the suggestion that female birds can manipulate yolk hormone levels independently of their own plasma levels or that yolk levels are somehow buffered as happens in viviparous lizards (Painter et al., 2002). However, Groothuis and Schwabl (2002) showed that although yolk testosterone varied about two fold with laying sequence in black-headed gulls (*Larus ridibundus*), variation among-individuals was much greater than this for any given laying sequence. Similarly, in European starlings, Pilz et al. (2003) showed that plasma androgens increased significantly with laying sequence but variation in yolk androgen levels was much greater between-females than within-females. The paradox therefore is that if relatively small intra-clutch differences in yolk steroid levels can have marked effects on offspring phenotype (Eising et al., 2001; Lipar and Ketterson, 2000; Schwabl, 1996a; Sockman and Schwabl, 2000) why are there not even more extreme effects on offspring phenotype due to the much larger variation in yolk hormones among individuals?

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References

Adkins-Regan, E., Ottinger, M.A., Park, J., 1995. Maternal transfer of estradiol to egg yolks alters sexual differentiation of avian offspring. J. Exp. Zool. 271, 466–470.

al-Afaleq, A.I., Homeida, A.M., 1998. Effects of low dose oestradiol, testosterone, and dihydrotestosterone on the immune response of broiler chicks. Immunopharmacol. Immunotoxicol. 20, 315–327.

Bahr, J.M., Wang, S.-C., Huang, M.Y., Calvo, F.O., 1983. Steroid concentrations in isolated theca and granulosa layers of preovulatory follicles during the ovulatory cycle of the domerstic hen. Biol. Reprod. 29, 326–334.

Balthazart, J., 1983. Hormonal correlates of behaviour. In: Farner, D.S., King, J.R., Parkes, K.C. (Eds.), Avian Biology. Academic Press, New York, pp. 221–365.

Birkhead, T., Schwabl, H., Burke, T., 2000. Testosterone and maternal effects—integreating mechanisms and function. TREE 15, 86–87.

Bowden, R.M., Ewert, M.A., Freedberg, S., Nelson, C.E., 2002. Maternally derived yolk hormones vary in follicles of the painted turtle, *Chrysemys picta*. J. Exp. Zool. 293, 67–72.

Brant, J.W.A., Nalbandov, A.V., 1956. Role of sex hormones in albumen secretion by the oviduct of chickens. Poult. Sci. 52, 692–700.

Challenger, W.O, Williams, T.D, Christians, J.K., Vézina, F., 2001. Follicular development and plasma yolk precursor dynamics through the laying cycle in the European starling (*Sturnus vulgaris*). Physiol. Biochem. Zool. 74, 356–365.

Christians, J.C., Williams, T.D., 2001. Intraspecific variation in reproductive physiology and egg quality in the European starling (*Sturnus vulgaris*). J. Avian Biol. 32, 31–37.

Clermont, C.P., Schrar, H., 1979. Effect of estrogen on rate of ^{59}Fe uptake by hematopoietic tissue in Japanese quail. Am. J. Physiol. 236, E342–E346.

Dawson, A., 1983. Plasma gonadal steroid levels in wild starlings (*Sturnus vulgaris*) during the annual cycle and in relation to the stages of breeding. Gen. Comp. Endocrinol. 49, 286–294.

Diamond, J., Hammond, K., 1992. The matches, achieved by natural selection between biological capacities and their natural loads. Experientia 48, 551–557.

Eising, C.M., Eikenaar, C., Schwabl, H., Groothuis, T.G.G., 2001. Maternal androgens in Black-headed Gull (*Larus ridibundus*) eggs: consequences for chick development. Proc. R. Soc. Lond. B Biol. Sci. 268, 839–846.

Etches, R.J., 1996. Reproduction in Poultry. CAB International, Oxford.

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

Gil, D., Graves, J., Hazon, N., Wells, A., 1999. Male attractiveness and differential testosterone investment in Zebra Finch eggs. Science 286, 126–128.

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

Hackl, R., Bromundt, V., Daisley, J., Kotrschal, K., 2003. Distribution and origin of steroid hormones in the yolk of Japanese quail (*Coturnix coturnix japonica*). J. Comp. Physiol. B 173, 327–331.

Hau, M., Wikelski, M., Soma, K.K., Wingfield, J.C., 2000. Testosterone and year-round territorial aggression in a tropical bird. Gen. Comp. Endocrinol. 117, 20–33.

Janzen, F.J., Wilson, M.E., Tucker, J.K., Ford, S.P., 2002. Experimental manipulation of steroid concentrations in circulation and in egg yolks of turtles. J. Exp. Zool. 293, 58–66.

Johnson, A., 2000. Reproduction in the female. In: Whittow, G.C. (Ed.), Sturkie's Avian Physiology. Academic Press, San Diego, pp. 569–596.

Leitner, G., Landsman, T., Blum, O., Zaltsmann, N., Heller, E.D., 1996. Effects of gonadal steroids and their antagonists on the humoral immune response of immune-selected broiler chicks. Poult. Sci. 75, 1373–1382.

Lipar, J.L., Ketterson, E.D., 2000. Maternally derived yolk testosterone enhances the development of the hatching muscle in the Red-winged Blackbird *Aegelaius phoeniceus*. Proc. R. Soc. Lond. B Biol. Sci. 267, 2005–2010.

Lipar, J.L., Ketterson, E.D., Nolan Jr., V., 1999. Intraclutch variation in testosterone content of Red-winged Blackbird eggs. Auk 116, 231–235.

Meijer, T., 1990. Incubation development and clutch size in the starling. Ornis Scand. 21, 163–168.

Mitchell, M.A., Carlisle, A.J., 1991. Plasma zinc as an index of vitellogenin production and reproductive status in the domestic fowl. Comp. Biochem. Physiol. 100A, 719–724.

Monroe, D.G., Jin, D.F., Sanders, M.M., 2000. Estrogen opposes the apoptotic effects of bone morphogenetic protein 7 on tissue remodeling. Mol. Cell Biol. 20, 4626–4634.

Muller, W., Eising, C.M., Dijikstra, C., Groothuis, T.G.G., 2002. Sex differences in yolk hormones depend on maternal status in Leghorn chickens (*Gallus gallus domesticus*). Proc. R. Soc. Lond. B Biol. Sci 269, 2249–2255.

Painter, D., Jennings, D.H., Moore, M.C., 2002. Placental buffering of maternal steroid hormone effects on fetal and yolk hormone levels: a comparative study of a viviparous lizard, *Sceloporus jarrovi*, and an oviparous lizard, *Sceloporus graciosus*. Gen. Comp. Endocrinol. 127, 105–116.

Pilz, K.M., Smith, H.G., Sandell, M.I., Schwabl, H., 2003. Interfemale variation in egg yolk androgen allocation in the European starling: do high-quality females invest more? Anim. Behav. 65, 841–850.

Redshaw, M.R., Follett, B.K., 1976. Physiology of egg yolk production by the fowl: the measurement of circulating levels of vitellogenin employing a specific radio-immunoassay. Comp. Biochem. Physiol. 55A, 399–405.

Salvante, K., Williams, T.D., 2002. Vitellogenin dynamics during egg-laying: daily variation, repeatability, and relationship with reproductive output. J. Avian Biol. 33, 391–398.

Sandell, M.I., Smith, H.G., 1997. Female aggression in the European starling during the breeding season. Anim. Behav. 53, 13–23.

SAS Institute, 1989. SAS/STAT User's Guide, Release 6.03 Edition. SAS Institute, Cary, NC.

Schultz, I.R., Orner, G., Merdink, J.L., Skilman, A., 2001. Dose–response relationships and pharmacokinetics of vitellogenin in rainbow trout after intravascular administration of 17α-ethynylestradiol. Aquat. Toxicol. 51, 305–318.

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

Schwabl, H., 1996a. Maternal testosterone in the avian egg enhances postnatal growth. Comp. Biochem. Physiol. 114A, 271–276.

Schwabl, H., 1996b. Environment modifies the testosterone levels of a female bird and its eggs. J. Exp. Zool. 276, 157–163.

Schwabl, H., Mock, D.W., Gieg, J.A., 1997. A hormonal mechanism for parental favouritisim. Nature 386, 231.

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

Sockman, K.W., Schwabl, H., 2001. Daily estradiol and progesterone levels relative to laying and onset of incubation in canaries. Gen. Comp. Endocrinol. 114, 257–268.

Vézina, F., Williams, T.D., 2002. Metabolic costs of egg production in the European Starling (*Sturnus vulgaris*). Physiol. Biochem. Zool. 75, 377–385.

Vézina, F., Williams, T.D., 2003. Plasticity in body composition in breeding birds: what drives the metabolic costs of egg production? Physiol. Biochem. Zool. 76, 713–730.

Wallace, R.A., 1985. Vitellogenesis and oocyte growth in nonmammalian vertebrates. In: Browder, L.W. (Ed.), Developmental Biology, vol. 1. Plenum Press, New York, pp. 127–177.

Walzem, R.L., Hansen, R.J., Williams, D.L., Hamilton, R.L., 1999. Estrogen induction of VLDLy assembly in egg-laying hens. J. Nutr. 129, 467S–472S.

Whittingham, L.A., Schwabl, H., 2002. Maternal testosterone in Tree Swallow eggs varies with female aggression. Anim. Behav. 63, 63–67.

Wikelski, M., Hau, M., Wingfield, J.C., 2000. Seasonality of reproduction in a neotropical rain forest bird. Ecology 81, 2458–2472.

Williams, T.D., 1999. Parental and first generation effects of exogenous 17β-estradiol on reproductive performance of female zebra finches (*Taeniopygia guttata*). Horm. Behav. 35, 135–143.

Williams, T.D., Ames, C.A., 2004. Top-down regression of the avian oviduct during late oviposition in a small passerine bird. J. Exp. Biol. 207, 263–268.

Williams, T.D., Martynuik, C.J., 1999. Tissue mass dynamics during egg-production in femal Zebra Finches *Taeniopygia guttata*: dietary and hormonal manipulations. J. Avian Biol. 31, 87–95.

Wingfield, J.C., Farner, D.S., 1975. The determination of five steroids in avian plasma by radioimmunoassay and competitive protein binding. Steroids 26, 311–327.

Yu, J.Y.-L., Campbell, L.D., Marquardt, R.R., 1971. Sex hormone control mechanisms. I. Effect of estrogen and progesterone on major cellular components in chicken (*Gallus domesticus*) oviducts. Can. J. Biochem. 49, 348–356.