Relationships between yolk androgens and nest density, laying date, and laying order in Western Burrowing Owls (*Athene cunicularia hypugaea*)

J.L. Welty, J.R. Belthoff, J. Egbert, and H. Schwabl

Abstract: Increases in yolk androgens within and among avian clutches have been correlated with decreased incubation time, increased aggression within a nest, increased begging behaviour, decreased immune response, and decreased life span. Although the mechanisms that lead to variability in yolk androgens within and between clutches are not completely known, yolk androgens can be a function of both social and environmental conditions. We were interested in if and how nesting density, laying date, and laying order influenced yolk androgens in Western Burrowing Owls (*Athene cunicularia hypugaea* (Bonaparte, 1825)) in which nest density varies considerably. In 2006 and 2007, we used radioimmunoassay to quantify the concentrations of testosterone, 5α-dihydrotestosterone, and androstenedione in the egg yolks from one early and one late-laid egg in 47 nests of Burrowing Owls located in the Morley Nelson Snake River Birds of Prey National Conservation Area in southern Idaho. Nesting density had no detectable effect on yolk androgens. Yolk androgens varied temporally and peaked in the middle of the laying season while being low before and after this time period. Within nests, late-laid eggs had higher testosterone and dihydrotestosterone than early-laid eggs; adrostendione exhibited a similar pattern in one but not both years of our study. It is possible that the seasonal pattern in yolk androgens that we observed is related to aspects of mate quality for females or declining chances of fledging success for later nesting females, whereas rises in egg androgens between early and late eggs within clutches could reflect a mechanism to assist nestlings from late-laid eggs that hatch one to several days after their siblings to better compete for resources within the nest or promote survival in the presence of larger siblings.

Résumé : On a établi une corrélation entre l'augmentation des androgènes du vitellus dans et entre les couvées d'oiseaux et la réduction du temps d'incubation, l'augmentation de l'agression dans le nid, l'augmentation du comportement de quête, la réduction de la réponse immunitaire et la réduction de la durée de vie. Bien que les mécanismes qui entraînent la variabilité des androgènes du vitellus dans les couvées et entre les couvées ne soient pas entièrement connus, les androgènes vitellins peuvent être fonction à la fois des conditions sociales et environnementales. Nous nous sommes intéressés à savoir si et comment la densité des nids, la date de ponte et l'ordre des pontes influencent les androgènes vitellins chez la chevêche des terriers de l'Ouest (*Athene cunicularia hypugaea* (Bonaparte, 1825)) dont la densité des nids est très variable. En 2006 et 2007, des tests radioimmunologiques nous ont permis de doser les concentrations de testostérone, de 5α-dihydrotestostérone et d'androsténédione dans le vitellus d'un œuf pondu tôt et d'un œuf pondu tard dans 47 nids de chevêches des terriers dans l'aire de conservation nationale des oiseaux de proie Morley Nelson Snake River dans le sud de l'Idaho. La densité de la nidification n'a aucun effet décelable sur les androgènes vitellins. Les androgènes vitellins varient dans le temps et atteignent un maximum au milieu de la période de ponte et sont réduits avant et après cette période. Au sein des couvées, les œufs pondus tard possèdent des concentrations plus élevées de testostérone et de dihydrotestostérone que les œufs pondus tôt; l'androsténédione suit un patron semblable durant l'une des années, mais pas durant les deux de notre étude. Il est possible que le patron saisonnier des androgènes vitellins observé soit relié à des aspects de qualité du partenaire chez les femelles ou alors à un déclin de la probabilité des oisillons nés plus tard à quitter le nid avec succès; par ailleurs, l'augmentation des androgènes vitellins entre les œufs hâtifs et tardifs dans les portées pourrait représenter un mécanisme pour aider les oisillons au nid des œufs tardifs, qui éclosent un à plusieurs jours après le reste de la fratrie, de mieux faire compétition pour les ressources au sein du nid ou pour favoriser leur survie en présence de frères et de sœurs de plus grande taille.

[Traduit par la Rédaction]

Received 27 March 2011. Accepted 7 November 2011. Published at www.nrcresearchpress.com/cjz on 31 January 2012.

J.L. Welty* and J.R. Belthoff. Boise State University, Department of Biological Sciences and Raptor Research Center, 1910 University Drive, Boise, ID 83725, USA.
J. Egbert and H. Schwabl. Washington State University, School of Biological Sciences, P.O. Box 644236, Pullman, WA 99164, USA.

Corresponding author: J.L. Welty (e-mail: justinwelty@gmail.com)

*Present address: USGS Forest and Rangeland Ecosystem Science Center, Snake River Field Station, 970 Lusk Avenue, Boise, ID 83706, USA.

Introduction

Variation in avian yolk androgens occurs among eggs of a single clutch, different clutches in the same area, different species in a given area, and similar species at different latitudes (Groothuis et al. 2005; Navara et al. 2006*a*; Martin and Schwabl 2008). These androgens function as regulatory signals during the differentiation of genotype into phenotype and mediators of phenotypic responses to environmental changes (reviewed by Groothuis and Schwabl 2008). Although proximate mechanisms associated with yolk androgen variation are only beginning to be understood, social and environmental factors acting on a female at the time of laying may influence levels in eggs (Whittingham and Schwabl 2002; Tanvez et al. 2008; Hargitai et al. 2009). Females may even be capable of fine tuning androgen content in an egg at the time of laying to maximize reproductive success given the current environmental conditions that they face (Pilz et al. 2004*a*). Factors that influence female and yolk androgens are relevant because increased egg androgen levels have been correlated with multiple trade-offs between costs and benefits including an increase in the size of the hatching muscle (Lipar and Ketterson 2000), accelerated hatching times and increased nestling growth rates (Eising et al. 2001), higher social rank likely resulting from increased aggression (Schwabl 1993), decreased nestling immune response (Navara et al. 2005), and reduced survival (Sockman and Schwabl 2000).

One factor that may alter yolk androgens is a high concentration of neighbouring conspecifics at the time of yolk production (Schwabl 1997; Whittingham and Schwabl 2002; Pilz and Smith 2004*b*). Eggs of laying females exposed to experimental territory intrusions can also contain higher yolk androgen concentrations (Hargitai et al. 2009). A parsimonious hypothesis is that similar to males (see Goymann et al. 2007), female aggressive or territorial interactions are mediated by an increase in circulating androgen levels, which is then reflected in the yolk. However, researchers generally find either no change or even a decrease in female circulating androgen levels during and after the expression of aggressive behaviour (Jawor et al. 2006; Navara et al. 2006*b*). Regardless of the mechanism, it is possible that higher yolk androgen levels give nestlings a competitive advantage (e.g., increased aggression) in densely populated environments (Pilz and Smith 2004*b*).

Yolk androgens may also vary seasonally. For example, clutches of European Starlings (*Sturnus vulgaris* L., 1758) laid earlier in the breeding season have greater concentration of testosterone (Pilz et al. 2003), whereas later clutches in Collared Flycatchers (*Ficedula albicollis* (Temminck, 1815)) have higher yolk testosterone (Michl et al. 2005). Moreover, Poisbleau et al. (2011) reported that laying date affected the pattern of androgen deposition into first and second eggs of Southern Rockhopper Penguins (*Eudyptes chrysocome chrysocome* (J.R. Forster, 1781)) in that late clutches had proportionally higher androgen levels in the B egg compared with the A egg than in early clutches. Thus, there may be species-specific seasonal effects on egg androgens.

In addition to potential variability in yolk androgen levels throughout the breeding season, yolk androgens often vary within a single clutch. While within clutch variation in egg androgens is not fully understood, it is often associated with hatching asynchrony (Müller et al. 2004; Tschirren et al. 2004; Hahn 2011). Hatching asynchrony often results in broods that show a hatching-order-dependent size hierarchy (Clark and Wilson 1985). Younger nestlings may be at both a size and competitive disadvantage against older, larger nestlings, such that they are less likely to survive to fledging (Mock et al. 1990). An increase in yolk androgens with laying order may help compensate for this disadvantage (Schwabl 1997; Tschirren et al. 2004; Hahn 2011). Increased egg androgens can promote overall growth of an embryo (Eising et al. 2001; Schwabl et al. 2007) or growth of the hatching muscle (Lipar 2001), both of which result in an earlier hatching date. Accelerated hatching allows for younger nestlings to be closer in size to their older siblings and, therefore, to more effectively compete for resources (Schwabl 1993; Eising and Groothuis 2003). Higher androgens may also alter nestling begging behaviour (e.g., Boncoraglio et al. 2006; von Engelhardt et al. 2006).

Despite such benefits, there are also potential costs of increased yolk androgens. Sockman and Schwabl (2000) demonstrated that artificially increasing androgens in first-laid eggs of American Kestrels (*Falco sparverius* L., 1758) delayed hatching, reduced overall body condition of nestlings, and caused higher nestling mortality. Artificially increased testosterone has also been linked to decreased immune response (Navara et al. 2005) and increased metabolic rate without increasing growth rate (Tobler et al. 2007). This requires the nestling to consume more resources to grow at the same rate as its siblings. Thus, natural selection may select for an optimum yolk androgen level for each egg.

Our objective was to examine the potential effects of (*i*) nesting density, (*ii*) laying date, and (*iii*) laying order on yolk testosterone (T), 5α-dihydrotestosterone (DHT), and androstenedione (A_4) to determine if and how variation in concentration of these androgens may be adaptive in Western Burrowing Owls (*Athene cunicularia hypugaea* (Bonaparte, 1825)). Burrowing Owls are an appropriate species in which to study these three factors because (*i*) nesting density varies, (*ii*) they are migrants in our study area and therefore potentially return and begin nesting at different times of the breeding season, (*iii*) large clutch sizes can result in a large amount of time between the first and last egg, and (*iv*) partial asynchronous hatching occurs.

Burrowing Owls typically nest in prairies, grasslands, steppes, and other open areas (Haug et al. 1993), including near irrigated agriculture in certain portions of their range (Conway et al. 2006; Moulton et al. 2006; Restani et al. 2008). They are opportunistic predators that feed on rodents, small birds, amphibians, reptiles, and a variety of invertebrates (Haug et al. 1993; Moulton et al. 2005). Burrowing Owls are socially monogamous, and females typically lay 8–12 eggs per clutch and incubate while their mates provision them (Haug et al. 1993). The laying period for a given female burrowing owl can last at least 2 weeks (Haug et al. 1993; Wellicome 2005). On average, pairs produce 0.9–4.9 fledglings per nesting attempt (Haug et al. 1993; Griebel and Savidge 2007).

Burrowing Owls nest in underground burrows that have been excavated by other animals such as American badgers (*Taxidea taxus* (Schreber, 1777)), prairie dogs (genus *Cyn-*

omys Rafinesque, 1817), and other burrowing mammals, although they also readily nest in artificial burrows placed by researchers (Smith and Belthoff 2001; Barclay 2008). These owls nest in both low-density (e.g., no nests within several kilometres of a focal nest) to high-density configurations (e.g., as many as 7 pairs nesting within a 400 m radius around a focal nest; Desmond and Savidge 1996; Moulton et al. 2005; Fisher et al. 2007), such that some investigators have used the term "colonies" to describe the denser distributions. In addition, both male and female Burrowing Owls interact aggressively with nearby conspecifics (Moulton et al. 2004; J.L. Welty, personal observation). Therefore, because of the potential for increasing aggressive interactions among females, increased nest density may increase yolk androgen levels.

In northern portions of their range, Burrowing Owls are generally present only during the breeding season (Haug et al. 1993). Although many Burrowing Owls that breed in Idaho appear to migrate, their migration routes and wintering areas remain poorly known (King and Belthoff 2001). However, upon return from wintering grounds, egg laying in Idaho generally begins in late March, peaks in April, and may continue through early May (J.L. Welty, personal observation). We were interested in whether and how this variability in laying date altered yolk androgens.

Finally, we examined the potential role of laying order in altering yolk androgens, as higher androgens in late-laid eggs frequently occurs in species with asynchronous hatching (Schwabl 1993; Tanvez et al. 2008). Age disparities between first- and last-hatched siblings (i.e., hatching spans) vary considerably (ranging between 1 and 7 days, mode of 4 days) in Burrowing Owls (Wellicome 2005), and this asynchronous hatching is frequently evidenced by size discrepancy in nestlings after all eggs have hatched. Thus, late-laid nestlings of Burrowing Owls may be at a competitive disadvantage, which altered yolk androgens could help them overcome.

Materials and methods

Field study protocols were approved by the Boise State University Institutional Care and Use Committee (Approval No. 006-05-013). From 1997 to 2007, we studied Burrowing Owls in and near the Morley Nelson Snake River Birds of Prey National Conservation Area (NCA) located in southwestern Idaho, USA. The NCA encompasses 195 325 ha, ~5% of which is irrigated agriculture. The agricultural areas grow primarily alfalfa, sugar beets, and mint. The remainder of the NCA is predominately shrub–steppe and grassland upon which some cattle and sheep grazing occurs, primarily during winter (USDI 1996). During the time of our study, there were ~300 artificial burrows in the NCA available for nesting and roosting by Burrowing Owls (Smith and Belthoff 2001; Belthoff and Smith 2003). Nesting pairs of Burrowing Owls occupied 30–60 of these artificial burrows per year (Belthoff and Smith 2003; J.R. Belthoff, unpublished data).

Beginning in late March of the 2006–2007 breeding seasons, we monitored artificial burrows once a week for the presence of adult Burrowing Owls. When we detected a mated pair, we inspected the nesting chamber in an artificial burrow to determine if the female had initiated egg laying and to determine initial laying date. To obtain samples for hormone analysis, we followed Schwabl (1993) and extracted ~75 mg of egg yolk using a 25-gauge, 1/2-inch (1 inch = 2.54 cm) butterfly needle to puncture the eggshell and enter the yolk. Yolk biopsies were repeated in the same manner for each egg with respect to egg position and needle depth in an attempt to sample from the same section of yolk in each egg. We swabbed eggs with alcohol both before and after the needle puncture to reduce risk of infection and sealed the puncture with either Loctite Super Glue Gel Control (Henkel Consumer Adhesives, Inc., Avon, Ohio, USA) or silicone (Window and Door Silicone II, Bioseal GE Sealants and Adhesives, Huntersville, North Carolina, USA). We transferred yolk samples into labeled centrifuge tubes stored on ice and, upon returning from the field, kept them frozen them at –20 °C until analysis.

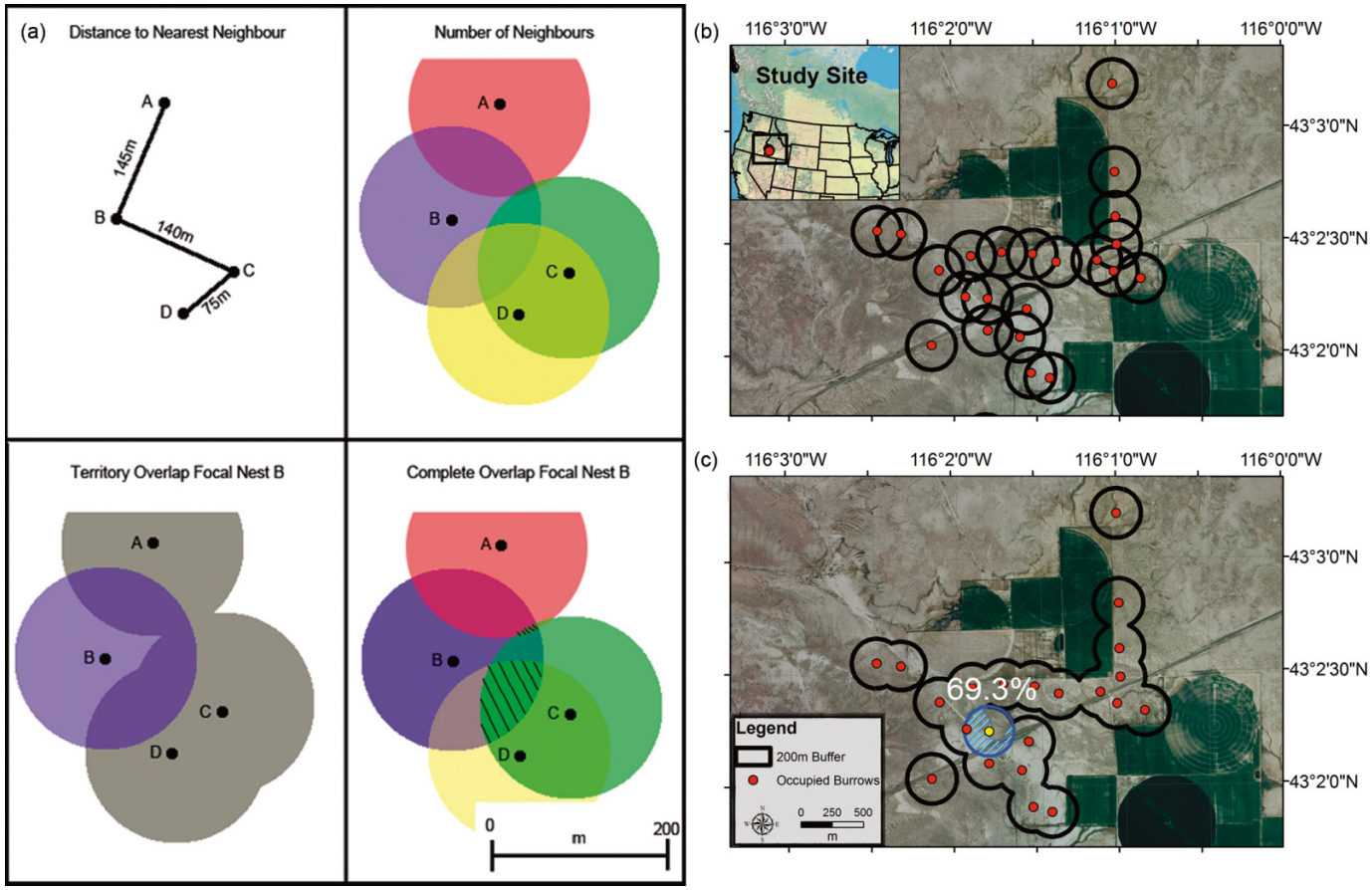
Nest density

We used ArcGIS version 9.2 (Environmental Systems Research Institute (ESRI), Inc., Redlands, California, USA) to calculate 10 separate measures of nest distribution as indices of nest density. These related to distance to nearest neighbour, number of nests within a focal buffer, and two separate measures of overlap of buffers surrounding nests (Figs. 1*a*–1*c*). Distance to nearest neighbour was the shortest straight-line distance between a focal nest and its neighbours. For number of nests and overlap, we first constructed buffers with a radius of 200, 400, and 600 m around each nest. Number of nests was the count of Burrowing Owl nests within each focal buffer. Overlap of buffers surrounding nests were calculated as (*i*) percent overlap, which was the percentage that a focal nest buffer was overlapped by any buffers from surrounding owl nests (0% to 100%), and (*ii*) complete overlap of a focal nest, which was the combined sum of each overlapping neighbouring nest buffer (0% to >100%; Figs. 1*a*–1*c*) at each buffer distance. Ultimately, however, these 10 density indices were strongly correlated (Spearman correlation: $r_S = |0.60–0.97|$, with all $p < 0.0001$). Therefore, we selected percent overlap with a 200 m buffer as our final index of nest density to be used in all subsequent analyses because Moulton et al. (2004) found that Burrowing Owls defend their territory in response to simulated territorial intrusion to distances of 100 m. Thus, 200 m represents a distance by which two nests can be separated and be predicted to have high levels of interaction. Importantly, inferences about the effects of nest density on yolk androgens were the same irrespective of the density index that we used in analyses.

Laying date

We sampled eggs from initial laying dates that spanned the breeding season and scored date as the integer day (0 representing 1 January and 365 representing 31 December) on which the female began to lay eggs. If laying had already begun upon first inspection, we back-calculated the initial laying date by estimating that one egg was laid every 1.5 days, as this has been the laying time frame within our study area (J.R. Belthoff, unpublished data). We only included nests where 1–4 eggs had been laid upon first inspection, which reduced need for extended extrapolation of initial laying date. For analysis, we adjusted laying date to the yearly median.

Fig. 1. Calculation of nest density indices for nests of Western Burrowing Owls (*Athene cunicularia hypugaea*) in 2006 and 2007 in the Morley Nelson Snake River Birds of Prey National Conservation Area (NCA), southwestern Idaho. (*a*) Note that for nest B, the closest neighbour is C, but C's closest neighbour is D. Number of neighbours counts the number of owls within a buffer around the nest. Percent overlap determines the total buffered area of a nest that other buffers overlap. Complete overlap is the sum of each neighbouring nest's overlap with the focal nest. The hatched areas indicate where more than one nest overlaps the same area, and this area is counted multiple times accordingly. (*b*) Locations of actual nests of Western Burrowing Owls within a portion of NCA with 200 m radius buffers. (*c*) A focal burrow has a nest density index of 69.3%.



Laying order

We were unable to determine exact laying order for all eggs within each nest of Burrowing Owls because to do so could have resulted in nest abandonment from the resulting disturbance. We also wanted to limit nest visits to decrease the possibility that our visits resulted in physiological changes in adult females that could alter yolk androgens (Poisbleau et al. 2009). Instead, we visited and inspected nests only with sufficient frequency (2–3 visits/nest) and at appropriate intervals so that we could divide eggs within a clutch into two categories that roughly corresponded to the first and second halves of a clutch. We refer to these as early and late eggs, respectively. We marked eggs on the shell with a small amount of dark ink to distinguish them as early or late. We obtained a yolk sample from one randomly selected early and one randomly selected late egg to determine if and how egg androgens differed within a clutch. We avoided sampling nests that showed obvious signs that incubation had begun (warm eggs or signs of colour change that come with incubation); in so doing, we ensured that we were measuring hormones that had initially accumulated in the yolks and not hormone concentrations altered through embryo growth (Gilbert et al. 2007).

Covariates

We also considered the influence of three potential covariates on yolk androgens: year, distance from agriculture, and female body condition. The study occurred over a 2-year period during which time environmental conditions could have varied, so we assessed year effects. As Burrowing Owls often associate with irrigated agriculture where the possibility of persistent organic pollutants could influence yolk hormone levels (Verboven et al. 2008; Poisbleau et al. 2009), we also measured distance to agricultural fields. Distance to irrigated agriculture was the straight-line distance between focal owl nests and the nearest irrigated agricultural field as measured by ArcGIS. We also calculated an index of female body condition at the time of capture, as female health may influence yolk hormones (Verboven et al. 2003). We indexed body condition using measurements of mass (g), wing length (mm), tail length (mm), and culmen length (mm) obtained at the time of female capture (usually 1–3 weeks after incubation commenced). As we did not capture females during the laying stage because of the potential for nest abandonment, our analyses assume that relative differences among females in body condition during the laying stage are maintained into the incubation stage. From these measurements, we cal-

culated female BCI (body condition index) using a principal components (PC) analysis of the size variables and regressing mass on scores along the first PC (index of size). The residuals from this regression were the BCI scores (Jakob et al. 1996), where positive residuals indicated owls in better body condition than expected based on size. We were unable to capture and measure the adult female at five nests, so we could not calculate BCI, which left a sample of 42 nests for some analyses. Ultimately, BCI was standardized by year for analysis.

Hormone assays

We performed radioimmunoassay using the modified Schwabl (1993) yolk hormone protocol. Yolk biopsies were homogenized in 300 μL of deionized water and ~2000 counts/min of tritiated A_4, DHT, and T were added to each sample to determine extraction efficiency. After equilibrating overnight at 4 °C, free steroids were extracted twice using 4 mL 30:70 (*v*/*v*) petroleum ether : diethyl ether. The samples were redissolved in 1 mL 90% ethanol and incubated at –20 °C overnight to precipitate neutral lipids. Samples were centrifuged at 1500 rev/min (400*g*) for 5 min at 4 °C, decanted into a new tube, and dried using compressed nitrogen in a 50 °C water bath. Samples were resuspended in 1 mL 10:90 (*v*/*v*) ethylacetate : isooctane (EA:IO). The solution was transferred to a glass column containing diatomaceous earth, dH_2O, and a 1:1 mixture of propylene glycol : ethylene glycol for hormone extraction. We used 4.0 mL of 2:98 EA:IO to extract A_4. DHT was extracted with 4.5 mL of 10:90 EA:IO. T was extracted using 4.5 mL of 30:70 EA:IO. Steroid fractions were dried in a 45 °C water bath and resuspended in 550 μL PBS with 0.1% gelatin (PBSg). Of this solution, 100 μL was used to quantify extraction efficiency and 100 μL duplicates (A_4 and T) or 200 μL duplicates (DHT) were used in the assays. The antibodies were T 3003 (Wien Laboratories) for T and DHT, and A 1707 (Wien Laboratories) for A_4. The labeled steroids were NET-533 for T, NET-544 for DHT, and NET 469 for A_4. We performed assays separately by year following each field season. Intra-assay variation for 2006/2007 samples was 6.0%/7.3% for T, 10.6%/12.6% for DHT, and 9.2%/9.8% for A_4. Minimum detectable levels were 0.249 pg/mg for T, 0.246 pg/mg for DHT, and 0.561 pg/mg for A_4.

Data analysis

We analyzed effects of nest density index, laying date, laying order (one early-laid egg versus one late-laid egg within a clutch), year, distance to agriculture, female BCI, and the lower order (i.e., two-way) interactions among these factors on log-transformed yolk androgens of Burrowing Owls using a mixed model approach in SAS version 9.2 (SAS Institute Inc. Cary, North Carolina, USA). Laying date had a curvilinear relationship with T, DHT, and A_4, so we fit an exponential term to better approximate this relationship. Laying order was modeled as a repeated measure in each case. We evaluated Akaike's information criterion corrected for small sample size (AIC_c) (Burnham and Anderson 2002) and examined *p* values on interaction terms to determine if main effects models or models including interactions were most appropriate. For both T and DHT, main effects models had either the lowest AIC_c score (T) or AIC_c scores that differed minimally from other models (DHT), and none of these models had interaction terms with significant *p* values; thus, we present results from main effects only models for T and DHT. For A_4, AIC_c was lowest for a model that included the year × laying order interaction, and examination of *p* values indicated this interaction term was significant. Therefore, for this interaction, A_4 means for early and late eggs were compared separately by year using *t* tests in the LSMEANS option on PROC MIXED. Means ± SE are presented unless otherwise indicated.

Results

We collected 48 yolk samples from 24 nests in 2006 and 46 samples from 23 nests in 2007. Yolk T, DHT, and A_4 averaged 10.8 ± 0.81 pg/mg yolk (range: 0.4–39.2 pg/mg), 8.4 ± 0.56 pg/mg yolk (range: 0.6–27.0 pg/mg), and 15.2 ± 1.24 pg/mg yolk (range: 0.7–62.4 pg/mg), respectively (n = 94 eggs from n = 47 nests in each case). T did not differ between years, but mean DHT was greater in 2006 (10.3 ± 0.92 pg/mg yolk, n = 48) than in 2007 (6.3 ± 0.42 pg/mg yolk, n = 46) (Table 1).

Nest density

Many nests of Burrowing Owls had nearest neighbours that were located within 250 m (median = 247 m, range: 84–3470 m, n = 47 nests). The index for nest density, i.e., overlap within a 200 m buffer, averaged 32.0% ± 3.27% (n = 46, range: 0.0%–95.9%). Despite substantial overlap values in many cases, we did not detect an effect of nest density on T, DHT, or A_4 concentrations in owl egg yolks (Table 1).

Laying date

The mean date on which owls laid their first egg was 15 April (integer day: 105 ± 1 days; range: 28 March to 12 May, n = 47 nests). The pattern of variation in T, DHT, and A_4 across the laying season was similar. The concentration of each hormone was low early in the season, peaked in mid-season, and declined as the end of the laying season approached. As such, all three androgens showed a significant curvilinear relationship with laying date (Table 1, Fig. 2).

Laying order

Yolk T and DHT were both significantly greater in late eggs (Table 1). T and DHT concentrations were 7.5 ± 1.01 pg/mg yolk (range: 0.4–39.2 pg/mg yolk) and 7.1 ± 0.70 pg/mg yolk (range: 0.6–25.2 pg/mg yolk) in early eggs (n = 47) and 13.9 ± 1.04 pg/mg yolk (range: 2.2–29.4 pg/mg yolk) and 9.6 ± 0.83 pg/mg yolk (range: 2.7–27.0 pg/mg yolk) in late eggs (n = 47), respectively. The same significant pattern of differences occurred between early and late eggs for A_4 but only in 2007, which gave rise to the significant interaction between year and laying order (Table 1) for A_4 (2007 Early: 7.9 ± 1.92 pg/mg yolk, range = 0.7–37.4 pg/mg yolk, n = 23; 2007 Late: 17.5 ± 2.21 pg/mg yolk, range = 3.7–46.0 pg/mg yolk, n = 23; $t_{[41]} = 5.88$, $P < 0.0001$; 2006 Early: 18.3 ± 2.34 pg/mg yolk, range = 3.0–43.1 pg/mg yolk, n = 24; 2006 Late: 16.4 ± 2.71 pg/mg yolk, range = 1.4–62.4 pg/mg yolk, n = 24; $t_{[41]} = 1.05$, $P = 0.30$). For T, DHT, and A_4, there was an absolute increase in egg yolk androgens from early to late eggs in 42,

Table 1. Results of three mixed model analyses to assess main variables contributing to variation in log-transformed levels of (*a*) testosterone (T), (*b*) 5α-dihydrotestosterone (DHT), and (*c*) androstenedione (A_4) in egg yolks from eggs of Western Burrowing Owls (*Athene cunicularia hypugaea*) from the Morley Nelson Snake River Birds of Prey National Conservation Area (NCA), southwestern Idaho.

	Estimate	SE	df	*t*	*p*
(*a*) T.					
Fixed effects					
Intercept	2.51	0.22	36	11.23	<0.001
Distance to agriculture	–0.00	0.00	36	1.14	0.26
Laying date	0.04	0.02	36	2.51	0.02
Laying date^2	–0.01	0.00	36	–3.41	<0.001
Nest density index	0.32	0.33	36	0.96	0.34
Female body condition index	0.09	0.12	36	0.77	0.45
Year	0.24	0.21	36	1.14	0.26
Laying order (early relative to late)	–0.95	0.14	42	–6.89	<0.001
Random effects					
Within-nest covariance	0.24	0.11			
Residual (overall error)	0.41	0.09			
(*b*) DHT.					
Fixed effects					
Intercept	2.13	0.17	36	12.30	<0.001
Distance to agriculture	–0.00	0.00	36	–0.61	0.55
Laying date	–0.00	0.01	36	–0.15	0.88
Laying date^2	–0.00	0.00	36	–2.52	0.02
Nest density index	0.18	0.09	36	–0.69	0.50
Female body condition index	0.00	0.01	36	0.62	0.54
Year	0.40	0.16	36	2.47	0.02
Laying order (early relative to late)	–0.41	0.10	42	–4.05	<0.001
Random effects					
Within-nest covariance	0.16	0.07			
Residual (overall error)	0.22	0.05			
(*c*) A_4.					
Fixed effects					
Intercept	2.85	0.25	36	11.55	<0.001
Distance to agriculture	0.00	0.00	36	0.11	0.92
Laying date	0.02	0.02	36	1.41	0.16
Laying date^2	–0.00	0.00	36	–2.71	0.01
Nest density index	0.00	0.00	36	0.11	0.91
Female body condition index	–0.15	0.12	36	–1.22	0.23
Year	–0.31	0.27	36	–1.15	0.26
Laying order (early relative to late)	–0.53	0.20	42	–2.72	0.01
Laying order × year	1.56	0.32	41	4.94	<0.001
Random effects					
Within-nest covariance	0.20	0.12			
Residual (overall error)	0.54	0.12			

35, and 29 of 47 nests, respectively. In only 3 (6.4%) nests were all three androgens higher in the early-laid egg.

Covariates

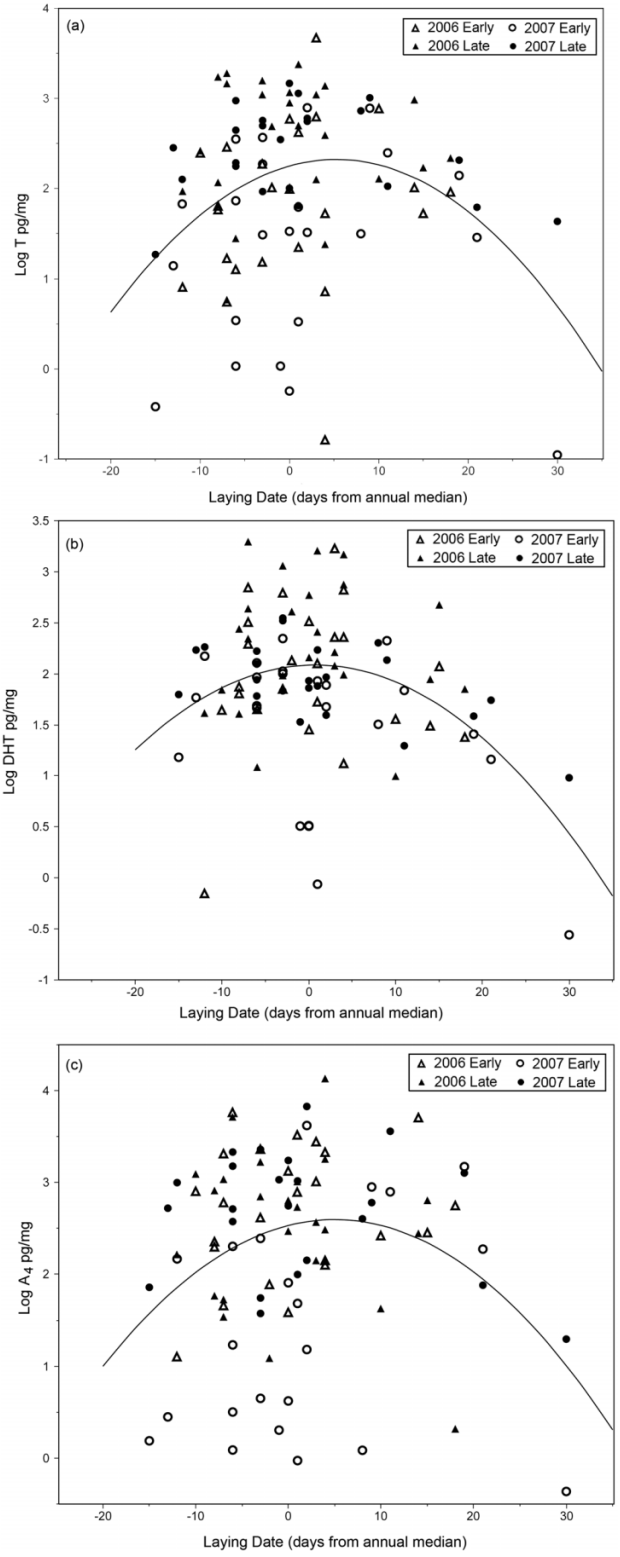
Distance to irrigated agriculture averaged 3.0 ± 0.41 km (median = 1.0 km, n = 47 nests, range: 0.1–12.9 km). Female body condition averaged 4.9 ± 1.9 (n = 42 adult females, range: –34.3 to 41.0). Neither distance to agriculture nor female body condition appeared to affect yolk androgens in eggs of Burrowing Owl (Table 1).

Discussion

Ours is one of only a small number of studies of yolk androgens in raptors and among the first reports in owls. We documented the consistent presence of three androgens in egg yolks of Burrowing Owls and evaluated three factors that might drive variability in their concentrations: nest density, laying date, and laying order. Our findings suggest that yolk androgens of Burrowing Owls do not correlate with nesting density but can vary both within a nest and among nests throughout the breeding season. Moreover, patterns of differences in yolk androgens between early and late eggs and through the breeding season remained substantially similar between years of study.

Despite relatively small nearest neighbour distances, substantial overlap of focal buffers around nests of Burrowing Owls, and marked variability in T, DHT, and A_4 levels

Fig. 2. Log-transformed levels of (*a*) testosterone (T), (*b*) 5α-dihydrotestosterone (DHT), and (*c*) androstenedione (A_4) in egg yolks of Western Burrowing Owls (*Athene cunicularia hypugaea*) in nests from 2006 and 2007 and early and late eggs in relationship to laying date in the Morley Nelson Snake River Birds of Prey National Conservation Area (NCA), southwestern Idaho (n = 94 eggs from 47 clutches).



among nests, there was no evidence of a nest density effect on yolk androgens of Burrowing Owls. One possible explanation is that neighbouring females did not interact with sufficiently high frequency or intensity to affect their hormonal milieu preceding or during the egg-laying period. For instance, nesting Burrowing Owls respond to simulated territorial intrusion with more aggressive approaches when intruders are within 50 m, but they respond more frequently with vocal displays when conspecifics are >100 m away (Moulton et al. 2004). Median distance to nearest nests was ~250 m, so neighbouring owls were sometimes more than 100 m away from one another. Thus, they may have responded simply with vocalizations rather than more aggressive behaviours (Moulton et al. 2004), while the latter may be needed to substantially alter yolk androgen levels. Alternatively, with documented extra-pair fertilizations in Burrowing Owls (Johnson 1997), this potential for extra-pair matings may select for behaviour on the part of males that protects paternity. Males may therefore engage in forms of mate guarding during the laying period, as occurs in other raptors (Mougeot 2004). Such behaviour by males could limit forays of females into other territories where aggressive interactions with residents could subsequently alter circulating hormone levels. Whatever the cause, our observational results indicate that egg androgens do not vary predictably with nest density in Burrowing Owls.

Additionally, neither distance of the nest to irrigated agricultural fields nor adult female BCI affected yolk androgens. Theoretically, there is potential for agricultural pesticides or other human activities in agricultural areas to alter hormones in adults and therefore affect their eggs (Verboven et al. 2008; Poisbleau et al. 2009). Despite such potential, we found no previous studies in birds that documented effects of agriculture or pesticides on egg androgens. However, the potential effects of pesticides on owls could vary with type of pesticide being used, relative persistence of the pesticides, and frequency of application, among other factors. This information is not available for owls nesting in the NCA because detailed pesticide records are not available from agricultural agencies. In addition to agriculture having no apparent effect, the lack of an adult female BCI effect in Burrowing Owls is similar to that reported by Pilz et al. (2003), who found no relationship between body condition and yolk androgens in European Starlings.

Burrowing Owls in the NCA initiated egg laying as early as late March and as late as mid-May. T, DHT, and A_4 in eggs of Burrowing Owls were typically low early in the season, mean levels peaked near mid-season, and levels declined toward the end of the nesting season. Verboven et al. (2003) found that egg androgens increased from early to late eggs within a clutch but documented no change with laying date in Lesser Black-backed Gulls (*Larus fuscus* L., 1758). In contrast, Pilz et al. (2003) observed higher egg yolk androgens in European Starlings earlier in the season perhaps because young that fledge early have a higher success rate, and females invested more by increasing yolk androgens in nestlings for which success is most likely. Michl et al. (2005) found Collared Flycatchers increase yolk androgens in clutches laid later in the season. They hypothesized that older males, who are better foragers, nest earlier in the season, and later nesting females must compensate for younger, inexper-

ienced males by increasing yolk androgens in those eggs which has been linked with increased nestling begging behaviour resulting in increased provisioning by the males (Eising et al. 2001). Finally, Poisbleau et al. (2011) demonstrated that laying order and laying date can interact to influence androgen levels. These studies suggest species-specific patterns in androgens in relation to laying date. We speculate that male Burrowing Owls that nest early in the year may be of higher quality, as they are able to migrate earlier in the season, and, as a result, females may produce eggs with lower androgens because the likelihood of fledging success is already high. Moreover, there is frequently a decline in the number of fledglings of Burrowing Owls per nest as the nesting season progresses (J.R. Belthoff, unpublished data). Consequently, females may have laid eggs with lower yolk androgens late in the season because of the decreased likelihood of success and possible poorer male quality.

There was clear evidence for greater concentrations of androgens in later laid eggs within individual clutches of Burrowing Owls. Finding a pattern of increasing yolk androgens with laying order has been common since Schwabl's (1993) first report on Atlantic Canaries (*Serinus canaria* (L., 1758)). For example, Sockman and Schwabl (2000) found an increase with laying order in American Kestrels. Additonally, Schmaltz et al. (2008) reported increases of T within clutches of Smooth-billed Anis (*Crotophaga ani* L., 1758), but they found no effect of density on T, which is similar to our observations in Burrowing Owls. In one of the only other published studies on yolk androgens in owls, Hahn (2011) found that only the first egg had lower androgen concentrations than the rest of the clutch in a captive population of Eastern Screech-Owls (*Megascops asio* (L., 1758)). How these results compare with free-living Screech-Owls is unknown, but it is similar to the Burrowing Owls that we studied in that a pattern of increasing androgen concentration between early and later eggs occurs. One factor, asynchronous hatching, appears to be a common element in many of the species for which late-laid eggs have higher yolk androgens (e.g., Schwabl 1993; Schmaltz et al. 2008; Tanvez et al. 2008). This is true for Burrowing Owls as well.

Even though it may appear to occur in a relatively safe environment, one of the most competitive periods in a bird's life may be the nestling period (Ros 2008). Increasing androgens may decrease incubation time in late-laid eggs (Eising et al. 2001; Schwabl et al. 2007) and increase competitiveness and food begging among the nestlings that hatch from them (Schwabl 1993; Eising and Groothuis 2003). This increase in aggression and begging can in turn allow younger nestlings who receive higher androgen levels to more effectively compete for the resources available within a nest (Schwabl 1993; Ros 2008; Müller and Eens 2009) and, in times of limited resources, the competition can be fierce. For example, if environmental conditions decrease prey populations, this in turn can impact predators, as there may not be sufficient resources to support all nests or all nestlings within a nest. Smith and Johnson (1985), in a 7-year study of Townsend ground squirrel (*Spermophilus townsendi* Bachman, 1839) in southern Idaho, found that in one drought year grass cover decreased from 14.9% to >1%, and this resulted in a 50% decline in the population of ground squirrels. Declines in prey density can have a direct negative effect on raptor density, breeding success, and fledgling survival (Rutz and Bijlsma 2006; Wiens et al. 2006; Sergio et al. 2008). Although Burrowing Owls feed mainly on rodents other than ground squirrels (Moulton et al. 2005, 2006), when rodent populations decline like this, younger nestlings of Burrowing Owls may gain some advantage from the physiological results of greater yolk androgens. On the other hand, later-hatched nestlings may act as a final food source for their older, stronger siblings. Rather than being compensatory, increased yolk androgens in later-laid eggs could facilitate brood reduction in poor food environments by potentially initiating a developmental program in these chicks that cannot be energetically supported. Under these circumstances, smaller nestlings may simply starve and be consumed by nest mates after their death, as we have observed portions of their remains in nest burrows with obvious signs that they have been fed upon by conspecifics. In contrast, when food is plentiful, higher androgen levels in later eggs may help these nestlings compete with older siblings for food within the nest and allow these nestlings to become competitive as adults.

In addition to the factors related to yolk hormone variability which our study addressed, it is likely that others contribute. For example, Gil et al. (2004, 2006) found greater amounts of yolk T in eggs of Zebra Finches (*Taeniopygia guttata* (Vieillot, 1817)) when the female was exposed to preferred male songs and in eggs of Barn Swallows (*Hirundo rustica* L., 1758) when mates had experimentally elongated tails. However, Navara et al. (2006*c*) found female House Finches (*Carpodacus mexicanus* (Statius Muller, 1776)) increased egg yolk androgen concentrations in eggs sired by less attractive males. Kingma et al. (2008) determined that experimentally altering a male's appearance to a more dominant state increased T in the subsequently laid eggs of their mates. It is likely that factors related to male quality also operate in Burrowing Owls, although this remains to be examined.

In summary, despite the potential for increased aggressive interactions among females nesting in areas of higher owl density, there was no apparent effect of nest density on yolk androgens in Burrowing Owls. Instead, variability in yolk androgens appeared to be a product of both the within-nest environment (laying order) and the external environment (laying date) in which the eggs were laid. Determining how variance in egg androgens differentially affects nestling development, behaviour, survival, and fitness in Burrowing Owls are important next steps.

Acknowledgements

Tscharntke et al. (2007) suggested to state the method of how the author sequence in a paper was assigned in the acknowledgements section: in this contribution we followed the sequence-determines-credit (SDC) approach, i.e., authors are listed according to the importance of their contribution to the work. This project was supported in part by Agriculture and Food Research Initiative Competitive Grant No. 2006-35101-17430 to J.R.B. from the USDA National Institute of Food and Agriculture Environment and Natural Resources Program, as well as by the Raptor Research Center and Department of Biological Sciences at Boise State University. We thank E. Dehamer, K. Ellsworth, K. McVey, C. Riding, M. Stuber, and L. Welty for assistance with fieldwork. We

especially thank L. Bond, who provided assistance with statistical analyses, and M. Fuller, Director of the Raptor Research Center, for their support. Finally, we thank I. Robertson, A. Dufty, Jr., and two anonymous reviewers for comments that very much improved the manuscript.

References

Barclay, J.H. 2008. A simple artificial burrow design for burrowing owl. J. Raptor Res. **42**(1): 53–57. doi:10.3356/JRR-06-85.1.

Belthoff, J.R., and Smith, B.W. 2003. Patterns of artificial burrow occupancy and reuse by burrowing owls in Idaho. Wildl. Soc. Bull. **31**: 138–144.

Boncoraglio, G., Rubolini, D., Romano, M., Martinelli, R., and Saino, N. 2006. Effects of elevated yolk androgens on perinatal begging behavior in yellow-legged gull (*Larus michahellis*) chicks. Horm. Behav. **50**(3): 442–447. doi:10.1016/j.yhbeh.2006.05.005. PMID:16842788.

Burnham, K.P., and Anderson, D.R. 2002. Model selection and multimodel inference: a practical information–theoretic approach. 2nd ed. Springer Science, New York.

Clark, A.B., and Wilson, D.S. 1985. The onset of incubation in birds. Am. Nat. **125**(4): 603–611. doi:10.1086/284365.

Conway, C.J., Garcia, V., Smith, M.D., Ellis, L.A., and Whitney, J.L. 2006. Comparative demography of Burrowing Owls in agricultural and urban landscapes in southeastern Washington. J. Field Ornithol. **77**(3): 280–290. doi:10.1111/j.1557-9263.2006.00054.x.

Desmond, M.J., and Savidge, J.A. 1996. Factors influencing burrowing owl (*Speotyto cunicularia*) nest densities and numbers in western Nebraska. Am. Midl. Nat. **136**(1): 143–148. doi:10.2307/2426639.

Eising, C.M., and Groothuis, T.G.G. 2003. Yolk androgens and begging behaviour in black-headed gull chicks: an experimental field study. Anim. Behav. **66**(6): 1027–1034. doi:10.1006/anbe.2003.2287.

Eising, C.M., Eikenaar, C., Schwabl, H., and 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**(1469): 839–846. doi:10.1098/rspb.2001.1594. PMID:11345330.

Fisher, J.B., Trulio, L.A., Biging, G.S., and Chromczak, D. 2007. An analysis of spatial clustering and implications for wildlife management: a burrowing owl example. Environ. Manage. **39**(3): 403–411. doi:10.1007/s00267-006-0019-y. PMID:17253092.

Gil, D., Heim, C., Bulmer, E., Rocha, M., Puerta, M., and Naguib, M. 2004. Negative effects of early developmental stress on yolk testosterone levels in a passerine bird. J. Exp. Biol. **207**(13): 2215–2220. doi:10.1242/jeb.01013. PMID:15159426.

Gil, D., Ninni, P., Lacroix, A., De Lope, F., Tirard, C., Marzal, A., and Møller, A.P. 2006. Yolk androgens in the barn swallow (*Hirundo rustica*): a test of some adaptive hypotheses. J. Evol. Biol. **19**(1): 123–131. doi:10.1111/j.1420-9101.2005.00981.x. PMID:16405584.

Gilbert, L., Bulmer, E., Arnold, K.E., and Graves, J.A. 2007. Yolk androgens and embryo sex: maternal effects or confounding factors? Horm. Behav. **51**(2): 231–238. doi:10.1016/j.yhbeh.2006.10.005. PMID:17187788.

Goymann, W., Landys, M.M., and Wingfield, J.C. 2007. Distinguishing seasonal androgen responses from male–male androgen responsiveness—revisiting the challenge hypothesis. Horm. Behav. **51**(4): 463–476. doi:10.1016/j.yhbeh.2007.01.007. PMID:17320880.

Griebel, R.L., and Savidge, J.A. 2007. Factors influencing burrowing owl reproductive performance in contiguous shortgrass prairie. J. Raptor Res. **41**(3): 212–221. doi:10.3356/0892-1016(2007)41[212:FIBORP]2.0.CO;2.

Groothuis, T.G., and Schwabl, H. 2008. Hormone-mediated maternal effects in birds: mechanisms matter but what do we know of them? Philos. Trans. R. Soc. Lond. B Biol. Sci. **363**(1497): 1647–1661. PMID:18048291.

Groothuis, T.G., Müller, W., von Engelhardt, N., Carere, C., and Eising, C. 2005. Maternal hormones as a tool to adjust offspring phenotype in avian species. Neurosci. Biobehav. Rev. **29**(2): 329–352. doi:10.1016/j.neubiorev.2004.12.002. PMID:15811503.

Hahn, D.C. 2011. Patterns of maternal yolk hormones in eastern screech owl eggs (*Megascops asio*). Gen. Comp. Endocrinol. **172**(3): 423–429. doi:10.1016/j.ygcen.2011.04.001. PMID:21510950.

Hargitai, R., Arnold, K.E., Herényi, M., Prechl, J., and Török, J. 2009. Egg composition in relation to social environmental and maternal physiological condition in the collard flycatcher. Behav. Ecol. Sociobiol. **63**(6): 869–882. doi:10.1007/s00265-009-0727-4.

Haug, E.A., Millsap, B.A., and Martell, M.S. 1993. Burrowing Owl (*Athene cunicularia*). *In* The birds of North America Online. *Edited by* A. Poole. Cornell Lab of Ornithology, Ithaca. Available from http://bna.birds.cornell.edu/bna/species/061/articles/introduction [accessed 10 October 2007].

Jakob, E.M., Marshall, S.D., and Uetz, G.W. 1996. Estimating fitness: a comparison of body condition indices. Oikos, **77**(1): 61–67. doi:10.2307/3545585.

Jawor, J.M., Young, R., and Ketterson, E.D. 2006. Females competing to reproduce: dominance matters but testosterone may not. Horm. Behav. **49**(3): 362–368. doi:10.1016/j.yhbeh.2005.08.009. PMID:16226754.

Johnson, B.S. 1997. Reproductive success, relatedness, and mating patterns of colonial burrowing owls. J. Raptor Res. **9**: 64–67.

King, R.A., and Belthoff, J.R. 2001. Post-fledging dispersal of Burrowing Owls in southwestern Idaho: characterization of movements and use of satellite burrows. Condor, **103**(1): 118–126. doi:10.1650/0010-5422(2001)103[0118:PFDOBO]2.0.CO;2.

Kingma, S.A., Komdeur, J., Vedder, O., von Engelhardt, N., Korsten, P., and Groothuis, G.C. 2008. Manipulation of male attractiveness induces rapid changes in avian maternal yolk androgen deposition. Behav. Ecol. **20**(1): 172–179. doi:10.1093/beheco/arn130.

Lipar, J.L. 2001. Yolk steroids and the development of the hatching muscle in nestling European Starlings. J. Avian Biol. **32**(3): 231–238. doi:10.1111/j.0908-8857.2001.320305.x.

Lipar, J.L., and Ketterson, E.D. 2000. Maternally derived yolk testosterone enhances the development of the hatching muscle in the red-winged blackbird *Agelaius phoeniceus*. Proc. R. Soc. Lond. B Biol. Sci. **267**(1456): 2005–2010. doi:10.1098/rspb.2000.1242. PMID:11075714.

Martin, T.E., and Schwabl, H. 2008. Variation in maternal effects and embryonic development rates among passerine species. Philos. Trans. R. Soc. Lond. B Biol. Sci. **363**(1497): 1663–1674. doi:10.1098/rstb.2007.0009. PMID:18048289.

Michl, G., Török, J.C., Péczely, P., Garamszegi, L.Z., and Schwabl, H. 2005. Female collared flycatchers adjust yolk testosterone to male age, but not to attractiveness. Behav. Ecol. **16**(2): 383–388. doi:10.1093/beheco/ari002.

Mock, D.W., Drummond, H., and Stinson, C.H. 1990. Avian siblicide. Am. Sci. **78**: 438–449.

Mougeot, F. 2004. Breeding density, cuckoldry risk and copulation behavior during the fertile period in raptors: a comparative analysis. Anim. Behav. **67**(6): 1067–1076. doi:10.1016/j.anbehav.2003.10.011.

Moulton, C.E., Brady, R.S., and Belthoff, J.R. 2004. Territory

defense of nesting Burrowing Owls: responses to simulated conspecific intrusion. J. Field Ornithol. **75**: 288–295.

Moulton, C.E., Brady, R.S., and Belthoff, J.R. 2005. A comparison of breeding season food habits of Burrowing Owls nesting in agricultural and nonagricultural habitat in Idaho. J. Raptor Res. **39**(4): 429–438.

Moulton, C.E., Brady, R.S., and Belthoff, J.R. 2006. Association between wildlife and agriculture: underlying mechanisms and implications in burrowing owls. J. Wildl. Manage. **70**(3): 708–716. doi:10.2193/0022-541X(2006)70[708:ABWAAU]2.0.CO;2.

Müller, W., and Eens, M. 2009. Elevated yolk androgen levels and the expression of multiple sexually selected male characters. Horm. Behav. **55**(1): 175–181. doi:10.1016/j.yhbeh.2008.09.012. PMID: 18976657.

Müller, W., Eising, C.M., Dijkstra, C., and Groothuis, T.G.G. 2004. Within-clutch patterns of yolk testosterone vary with the onset of incubation in black-headed gulls. Behav. Ecol. **15**(6): 893–897. doi:10.1093/beheco/arh091.

Navara, K.J., Hill, G.E., and Mendonça, M.T. 2005. Variable effects of yolk androgens on growth, survival, and immunity in eastern bluebird nestlings. Physiol. Biochem. Zool. **78**(4): 570–578. doi:10.1086/430689. PMID:15957111.

Navara, K.J., Hill, G.E., and Mendonça, M.T. 2006*a*. Yolk testosterone stimulates growth and immunity in house finch chicks. Physiol. Biochem. Zool. **79**(3): 550–555. doi:10.1086/501054. PMID:16691520.

Navara, K.J., Siefferman, L.M., Hill, G.E., and Mendonça, M.T. 2006*b*. Yolk androgens vary inversely to maternal androgens in eastern bluebirds: an experimental study. Funct. Ecol. **20**(3): 449–456. doi:10.1111/j.1365-2435.2006.01114.x.

Navara, K.J., Hill, G.E., and Mendonça, M.T. 2006*c*. Yolk androgen deposition as a compensatory strategy. Behav. Ecol. Sociobiol. **60**(3): 392–398. doi:10.1007/s00265-006-0177-1.

Pilz, K.M., and Smith, H.G. 2004*b*. Egg yolk androgen levels increase with breeding density in European Starling, *Sturnus vulgaris*. Funct. Ecol. **18**(1): 58–66. doi:10.1111/j.1365-2435.2004.00811.x.

Pilz, K.M., Smith, H.G., Sandell, M.I., and Schwabl, H. 2003. Interfemale variation in egg yolk androgen allocation in the European starling: do high-quality females invest more? Anim. Behav. **65**(4): 841–850. doi:10.1006/anbe.2003.2094.

Pilz, K.M., Quiroga, M., Schwabl, H., and Adkins-Regan, E. 2004*a*. European starling chicks benefit from high yolk testosterone levels during a drought year. Horm. Behav. **46**(2): 179–192. doi:10.1016/j.yhbeh.2004.03.004. PMID:15256308.

Poisbleau, M., Demongin, L., Trouve, C., and Quillfeldt, P. 2009. Maternal deposition of yolk corticosterone in clutches of southern rockhopper penguins (*Eudyptes chrysocome chrysocome*). Horm. Behav. **55**(4): 500–506. doi:10.1016/j.yhbeh.2009.02.002. PMID: 19232349.

Poisbleau, M., Demongin, L., Chastel, O., Eens, M., and Quillfeldt, P. 2011. Yolk androgen deposition in rockhopper penguins, a species with reversed hatching asynchrony. Gen. Comp. Endocrinol. **170**(3): 622–628. doi:10.1016/j.ygcen.2010.11.027. PMID: 21130090.

Restani, M., Davies, J.M., and Newton, W.E. 2008. Importance of agricultural landscapes to nesting burrowing owls in the northern Great Plains, USA. Landsc. Ecol. **23**: 977–987. doi:10.1007/s10980-008-9259-y.

Ros, A.F.H. 2008. Patterns of testosterone responsiveness and immunity in relation to competitive behavior in chicks. Horm. Behav. **54**(2): 234–237. doi:10.1016/j.yhbeh.2008.02.022. PMID: 18417128.

Rutz, C., and Bijlsma, R.G. 2006. Food limitation in a generalist predator. Proc. R. Soc. Lond. B Biol. Sci. **273**(1597): 2069–2076. doi:10.1098/rspb.2006.3507. PMID:16846915.

Schmaltz, G., Quinn, J.S., and Schoech, S.J. 2008. Do group size and laying order influence maternal deposition of testosterone in smooth-billed ani eggs? Horm. Behav. **53**(1): 82–89. doi:10.1016/j.yhbeh.2007.09.001. PMID:17942099.

Schwabl, H. 1993. Yolk is a source of maternal testosterone for developing birds. Proc. Natl. Acad. Sci. U.S.A. **90**(24): 11446–11450. doi:10.1073/pnas.90.24.11446. PMID:8265571.

Schwabl, H. 1997. The contents of maternal testosterone in house sparrows *Passer domesticus* eggs vary with breeding conditions. Naturwissenschaften, **84**(9): 406–408. doi:10.1007/s001140050418.

Schwabl, H., Palacios, M.G., and Martin, T.E. 2007. Selection for rapid embryo development correlates with embryo exposure to maternal androgens among passerine birds. Am. Nat. **170**(2): 196–206. doi:10.1086/519397. PMID:17874371.

Sergio, F., Marchesi, L., and Pedrini, P. 2008. Density, diet and productivity of Long-eared Owls *Asio otus* in the Italian Alps: the importance of *Microtus* voles. Bird Study, **55**(3): 321–328. doi:10.1080/00063650809461538.

Smith, B.W., and Belthoff, J.R. 2001. Effects of nest dimensions on use of artificial burrow systems by burrowing owls. J. Wildl. Manage. **65**(2): 318–326. doi:10.2307/3802911.

Smith, G.W., and Johnson, D.R. 1985. Demography of a Townsend ground squirrel population in southwestern Idaho. Ecology, **66**(1): 171–178. doi:10.2307/1941317.

Sockman, K.W., and Schwabl, H. 2000. Yolk androgens reduce offspring survival. Proc. R. Soc. Lond. B. Biol. Sci. **267**(1451): 1451–1456. doi:10.1098/rspb.2000.1163. PMID:10983830.

Tanvez, A., Parisot, M., Chastel, O., and Leboucher, G. 2008. Does maternal social hierarchy affect yolk testosterone deposition in domesticated canaries? Anim. Behav. **75**(3): 929–934. doi:10.1016/j.anbehav.2007.08.006.

Tobler, M., Nilsson, J.-Å., and Nilsson, J.F. 2007. Costly steroids: egg testosterone modulates nestling metabolic rate in the zebra finch. Biol. Lett. **3**(4): 408–410. doi:10.1098/rsbl.2007.0127. PMID:17456447.

Tscharntke, T., Hochberg, M.E., Rand, T.A., Resh, V.H., and Krauss, J. 2007. Author sequence and credit for contributions in multiauthored publications. PLoS Biol. **5**(1): e18. doi:10.1371/journal.pbio.0050018. PMID:17227141.

Tschirren, B., Heintz, R., and Schwabl, H. 2004. Ectoparasite-modulated deposition of maternal androgens in great tit eggs. Proc. R. Soc. Lond. B Biol. Sci. **271**(1546): 1371–1375. doi:10.1098/rspb.2004.2730. PMID:15306335.

USDI (U.S. Department of the Interior). 1996. Effects of military training and fire in the Snake River Birds of Prey National Conservation Area. BLM/IDARNG Research Project Final Report. U.S. Geological Survey, Biological Research Division, Snake River Field Station, Boise, Idaho.

Verboven, N., Monaghan, P., Evans, D.M., Schwabl, H., Evans, N., Whitelaw, C., and Nager, R.G. 2003. Maternal condition, yolk androgens and offspring performance: a supplemental feeding experiment in the lesser black-backed gull (*Larus fuscus*). Proc. R. Soc. Lond. B Biol. Sci. **270**(1530): 2223–2232. doi:10.1098/rspb.2003.2496. PMID:14613608.

Verboven, N., Verreault, J., Letcher, R.J., Gabrielsen, G.W., and Evans, N.P. 2008. Maternally derived testosterone and 17β-estradiol in the eggs of Arctic-breeding glaucous gulls in relation to persistent organic pollutants. Comp. Biochem. Physiol. C Toxicol. Pharmacol. **148**: 143–151. doi:10.1016/j.cbpc.2008.04.010.

von Engelhardt, N., Carere, C., Dijkstra, C., and Groothuis, T.G.G. 2006. Sex-specific effects of yolk testosterone on survival,

begging and growth of zebra finches. Proc. R. Soc. Lond. B Biol. Sci. **273**(1582): 65–70. doi:10.1098/rspb.2005.3274. PMID: 16519236.

Wellicome, T.I. 2005. Hatching asynchrony in Burrowing Owls is influenced by clutch size and hatching success but not by food. Oecologia (Berl.), **142**(2): 326–334. doi:10.1007/s00442-004-1727-8. PMID:15480800.

Whittingham, L.A., and Schwabl, H. 2002. Maternal testosterone in tree swallow eggs varies with female aggression. Anim. Behav. **63**(1): 63–67. doi:10.1006/anbe.2001.1889.

Wiens, J.D., Noon, B.R., and Reynolds, R.T. 2006. Post-fledging survival of Northern Goshawks: the importance of prey abundance, weather, and dispersal. Ecol. Appl. **16**(1): 406–418. doi:10.1890/04-1915. PMID:16705989.