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Functional significance of variation in egg-yolk androgens in the American coot

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Abstract Maternally derived hormones in cleidoic eggs have been implicated in mediating growth, behavior, and social interactions among offspring. Given these widespread and significant effects, hormonal investments have the potential to greatly influence fitness of offspring. Intraspecific variation can exist at three levels (within individual eggs, among eggs within clutches, and among eggs from different females), each of which has different implications for offspring. We characterized all three levels of variation in maternally derived androgens (testosterone and androstenedione) present in yolks of American coot eggs. We found no variation in testosterone levels within eggs which suggests that embryos are exposed to constant androgen levels during development, and that field-based yolk biopsies are an appropriate way to sample eggs for this species. Within clutches, early-laid eggs had higher androgen levels than late-laid eggs, and this pattern may exacerbate negative effects of hatching asynchrony on chicks from late-hatching eggs if androgens provide chicks with a behavioral or growth advantage over chicks from eggs with lower androgen levels. American coots lay large clutches, and unequal resource allocation among offspring may be the optimal strategy for females with access to limited resources. Most of the variation in androgen levels occurred among eggs from different females. Females nesting on ponds with two other pairs laid eggs with significantly higher androgen levels than females living on ponds with fewer pairs. This suggests that increased territory defense behaviors influence the levels of androgens allocated to eggs and may be one mechanism underlying density-dependent effects on reproduction.

Keywords Egg-yolk androgens · Maternal effects · Intraspecific variation · Hatching asynchrony · Density-dependent effects

Introduction

Females can greatly influence the quality and performance of their offspring through non-genetic contributions to young during development. The ubiquity of developmental maternal effects and their pervasive influence on offspring performance after development has become more widely recognized (e.g., Lombardi 1996; Collazo 1996; Clark and Galef 1998). Maternal effects can have complex consequences for the evolution of both offspring and maternal traits (e.g., Wolf et al. 1998). In part, these complexities arise because maternal investments are traits shared by both females and offspring. For example, egg size is a trait that describes a female reproductive character as well as the developmental environment experienced by offspring. Because these traits are shared, the consequences of maternal effects can cascade to the next generation and influence offspring performance well after the interval of dependence on parents.

Studies from a wide variety of oviparous vertebrates indicate that eggs contain significant and measurable amounts of maternally derived hormonal resources (fish: Schreck et al. 1991; McCormick 1998, 1999; reptiles: Stamps 1994; Conley et al. 1997; Janzen et al. 1998; birds: Schwabl 1993; Adkins-Regan et al. 1995; McNabb and Wilson 1997; Schwabl et al. 1997; Lipar et al. 1999a, 1999b; Eising et al. 2001; Sockman and Schwabl 2000). In the context of the evolution of life histories, transmission of hormones between mother and offspring is particularly interesting because hormones can have multiple effects on a wide variety of morphological, physiological and behavioral traits (Ketterson and Nolan 1999).

A complete understanding of the adaptive significance of yolk androgens provided by females requires

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determining how yolk androgen levels affect variation in offspring performance, as well as identifying the proximate and ultimate causes that generate and maintain variation in hormone levels. Androgen levels in canary eggs (*Serinus canaria*) can have positive post-hatching effects on offspring begging behaviors, growth, and social dominance among siblings (Schwabl 1993, 1996). These offspring traits may be most important in mediating competitive interactions among siblings, and thus the patterns of yolk androgen levels within clutches may have the most serious consequences for offspring survival and brood reduction strategies. However, distinct patterns of yolk androgen levels also exist both within individual eggs and among different females (Lipar et al. 1999a, 1999b), and the potential adaptive significance of these patterns is not so clear.

The American coot (*Fulica americana*) is a ubiquitous and conspicuous waterbird that nests on wetlands throughout central and northern North America. Pairs of coots are socially monogamous and establish all-purpose breeding territories that are vigorously defended against conspecifics on the same pond (Gullion 1953). Females lay 1 egg per day and can lay up to 16 eggs per breeding attempt (authors, personal observations). Both males and females incubate eggs and start incubating prior to clutch completion, which results in hatching asynchrony and large age differences among siblings (Alisauskas and Arnold 1994). Here, we characterize patterns of variation in yolk androgens within eggs, among eggs within clutches, and among eggs from different females in a free-living population of American coots. We discuss the potential consequences of these patterns of variation on offspring and female performance and explore the role of endogenous and exogenous factors generating the observed patterns. We propose that social interactions among coot pairs during territory establishment and defense are a proximate cause responsible for the patterns of variation in yolk androgens among females, and that the existing pattern of yolk androgen levels within clutches contributes to brood reduction along with the effects of hatching asynchrony.

Materials and methods

Variation in hormone resources in eggs can exist at multiple levels: among species, among individual females within species, among eggs within a clutch, and within individual eggs. We characterized three levels of variation in egg-yolk androgens from a population of American coots breeding in the prairie-parkland region south of Minnedosa, Manitoba, Canada (50°16'N, 99°50'W) during the 1998 breeding season (May–June).

For analysis of variation within and among clutches we collected yolk samples from all eggs of 24 clutches (216 eggs). All nests present on a pond were located and marked, nests from which yolk samples were taken were visited daily, and yolk samples from all eggs were collected within 24 h of eggs being laid. For each nest we knew how many other pairs of coots (0, 1, or 2 other pairs) were also nesting on that pond. We collected yolk samples by first cleaning the egg shell surface with an alcohol swab (70% ethanol), inserting a 23-gauge needle through the egg-shell at the middle of the long axis of the egg, and drawing a small

sample (approximately 60 mg) of yolk with a 3 ml disposable syringe. We sealed the insertion hole with Crazy Glue Gel (The Borden Company, Brampton, Ontario), measured the greatest width and length of the egg to the nearest 0.1 mm with digital callipers and returned the egg to its nest. Upon return to the lab, we transferred yolk samples into 1.5-ml Eppendorf tubes and froze them at –20°C until extraction. Testosterone was measured in all yolk samples from all 24 clutches, and androstenedione was measured in yolk samples in all eggs from 18 of these clutches.

For analysis of within-egg variation in testosterone concentrations, we sampled seven unincubated eggs that were collected from a population of American coots breeding in north-east South Dakota (45°25'N, 97°22'W) in 1996 and frozen whole (–20°C) until analysis in 1998. We assayed three yolk samples from each egg, one at each of three layers (interior, intermediate and exterior) according to the methods of Lipar et al. (1999b).

When trying to detect patterns of hormone concentrations among or within yolks, it is important to eliminate variation in concentrations due to water content of yolks. The water content of fresh coot yolks ranges between 47% and 55% (mean water content is 49%), which can result in as much as a 19% error in measured levels assayed from wet yolks. We dried yolk samples to a constant mass before analysis to minimize effects of variation in water content through a combination of vacuum drying and exposure to air in a frost-free freezer. Once samples were dry, we weighed a sub-sample (approximately 30 mg) to the nearest 0.01 mg, added 400 µl distilled water and three glass beads to each sample and agitated them repeatedly to resuspend the yolk. We added 2000 cpm [³H]testosterone to all samples 12 h prior to extraction to allow for calculation of recoveries following extraction and chromatography procedures.

The samples were extracted twice with 4 ml petroleum and diethyl ether (30%:70%), snap-frozen and dried under N₂. Following extraction, we added 1 ml 90% ethanol to precipitate lipids, then centrifuged and evaporated the ethanol supernatant under N₂ (Schwabl 1993). We resuspended the extracts in 0.5 ml 10% ethyl acetate in 2-2-4 trimethyl pentane (iso-octane). These samples were run through chromatography columns that consisted of a celite:ethylene glycol:propylene glycol (6 g:1.5 ml:1.5 ml) upper phase and a celite:water (3 g:2 ml) lower phase. We ran four successive organic fractions of 4 ml through each column: 100% iso-octane fraction, and 2%, 10% and 20% fractions of ethyl acetate in iso-octane. Androstenedione was collected from the phase eluted with 2% ethyl acetate in iso-octane, and testosterone was collected from the phase eluted with 20% ethyl acetate in iso-octane. A sub-sample of the testosterone fraction was counted to calculate the recovery of radiolabeled testosterone. After separation, hormone levels were measured by competitive binding radioimmunoassay using kits with iodinated hormone and specific antibodies for both testosterone and androstenedione (Diagnostic Systems Laboratories, Webster, Tx., USA). Samples were run in duplicate and compared to a standard curve that ranged in concentration from 0.05 to 25 ng ml⁻¹ for testosterone (Fig. 1) and 0.01 to 10 ng ml⁻¹ for androstenedione. Yolk samples that were serially diluted with distilled water yielded a displacement curve that was parallel to the serial dilution of the testosterone standard (Fig. 1). Recoveries for testosterone ranged from 22% to 91% and averaged 65% (SE=0.8%). Androstenedione recovery was not measured directly; we assumed that androstenedione was recovered from the samples in the same proportion as the recovery of testosterone. Samples with recoveries less than 25% (*n*=3) were excluded from further analyses. The intra-assay coefficient of variation, based on duplicate samples, averaged 10% for testosterone and 13% for androstenedione. The inter-assay variation among the three testosterone assays and two androstenedione assays was calculated as the coefficient of variation among samples of a coot yolk pool that was run in all assays, and was 13% for testosterone and 21% for androstenedione. All samples measuring within-egg variation in testosterone levels were run in the same assay.

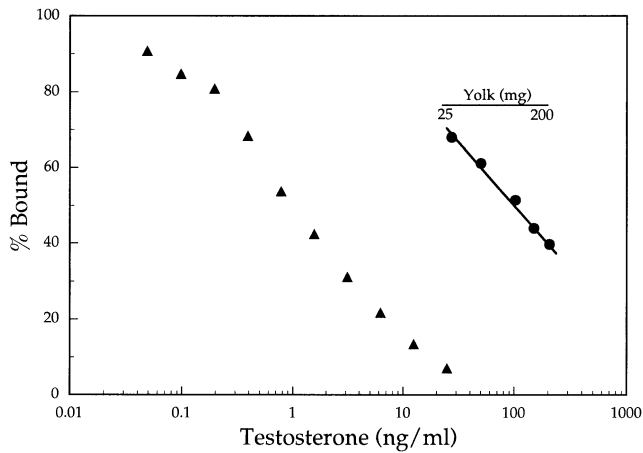


Fig. 1 Representative standard curve from a yolk testosterone radioimmunoassay. This graph shows the relationship between percentage binding of radiolabeled testosterone to testosterone antiserum and the concentration of testosterone (ng ml^{-1}). Also shown is the mean displacement curve of serially diluted yolk samples from two coot eggs

Statistics

We used ANOVA to test independently for variation in hormone concentration at three levels; within eggs, among eggs within clutches, and among clutches from different females. Mean hormone levels for females varied considerably among females; thus we standardized egg hormone levels by using the absolute deviation of each egg's hormone level from the mean hormone level for its clutch. This standardization sets the mean hormone level (in pg mg^{-1} dry yolk) for each female equal to zero and effectively takes into account variation among females and allows for a straight-forward analysis of variation within females. We used these deviations to test for differences in the mean hormone levels among eggs at different positions in the laying sequence (within-female variation in androgen levels). An alternative and analogous approach would be to use a repeated measured analysis with eggs as repeated measures within females. However, a repeated measures analysis is inappropriate in this case because coots lay a variable number of eggs and this results in an unbalanced design.

We tested for effects of female and the number of coot pairs nesting per pond on the logarithm of yolk androgen levels using a nested ANOVA with the number of pairs, and females nested within the number of pairs as main effects. Androgen levels for each egg were logarithmically transformed to normalize the data. We used Tukey-Kramer HSD as an a posteriori test of differences in mean androgen levels among females that nested on ponds with different numbers of pairs. Data were analyzed with JMP (SAS Institute, 1993) and are expressed as means \pm SE.

Results

Testosterone levels in egg yolks ranged from 0.2 to 6.1 pg mg^{-1} dry yolk (mean: 1.5 ± 0.1) and androstenedione concentrations ranged from 19 to 2775 pg mg^{-1} dry yolk (mean: 514 ± 33). Androstenedione levels were positively correlated with testosterone levels ($r=0.84$, $F_{1,163}=403$, $P<0.0001$), although androstenedione concentrations were nearly 100 times greater than testosterone concentrations in the same eggs (Fig. 2). Testosterone levels in the interior, intermediate and exterior layers averaged 1.7 ± 0.3 , 1.8 ± 0.3 and 1.7 ± 0.3 pg mg^{-1} dry yolk

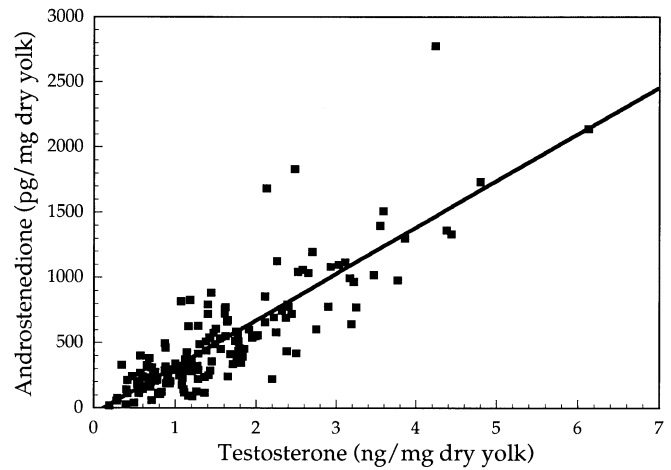


Fig. 2 Correlation between androstenedione and testosterone levels (pg mg^{-1} dry yolk) in American coot eggs. Linear equation describing the line: $\text{Androstenedione} = -49.60 + 358.63 \times \text{Testosterone}$ ($r=0.84$)

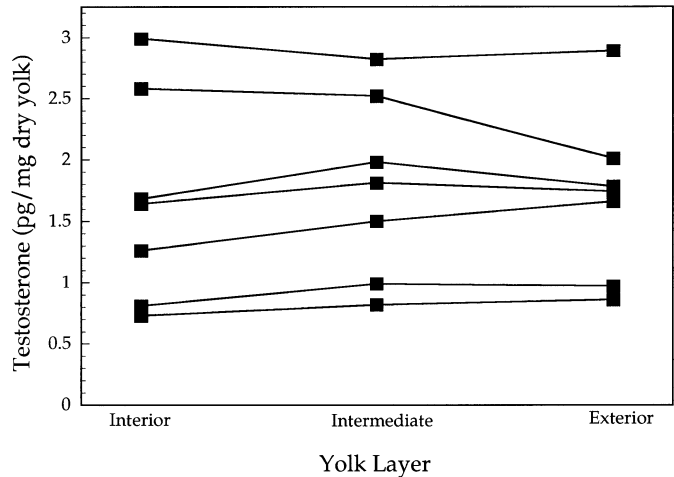


Fig. 3 Testosterone concentrations in three layers of yolks from 7 American coot eggs. There is no consistent pattern of variation among layers within eggs, but significant variation exists among the different eggs

respectively. Testosterone levels did not differ among sampling positions within the yolk ($F_{2,12}=0.75$, $P=0.49$), but did vary considerably among individual eggs ($F_{6,12}=59$, $P<0.0001$) (Fig. 3).

Within clutches, testosterone levels varied with laying sequence ($F_{10,209}=3.55$, $P=0.0002$). Clutch sizes ranged from 5 to 15 eggs, but only three clutches had more than 11 eggs, so we excluded eggs from lay positions greater than 11 from the analysis. Eggs laid late in the sequence had lower testosterone levels than eggs laid early in the sequence (Fig. 4). Likewise, androstenedione concentrations varied with laying sequence ($F_{10,159}=2.90$, $P=0.0024$) and showed a similar pattern to that of testosterone (Fig. 4).

Most of the variation in yolk steroid levels was due to differences among clutches produced by different fe-

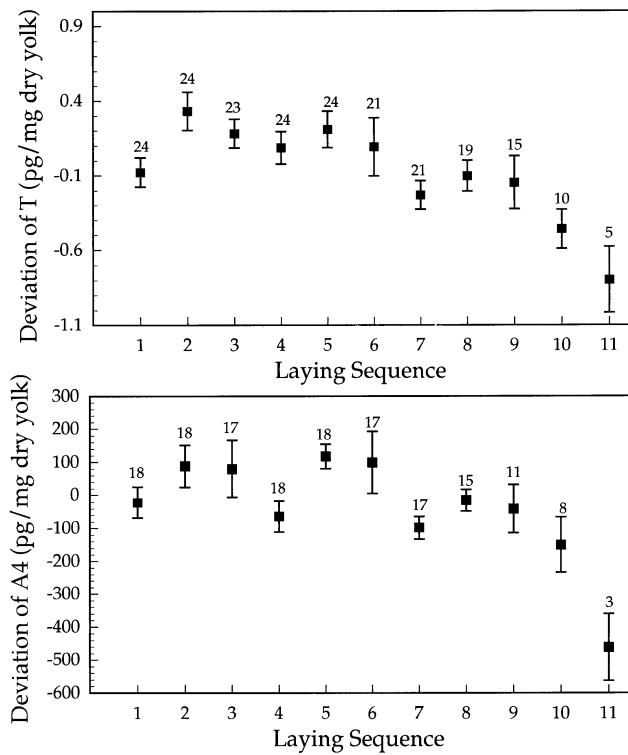


Fig. 4 Mean deviation of testosterone (*T*) and androstenedione (*A4*) levels in egg yolks from clutch means as a function of laying order. Numbers above error bars indicate the number of eggs in each laying position

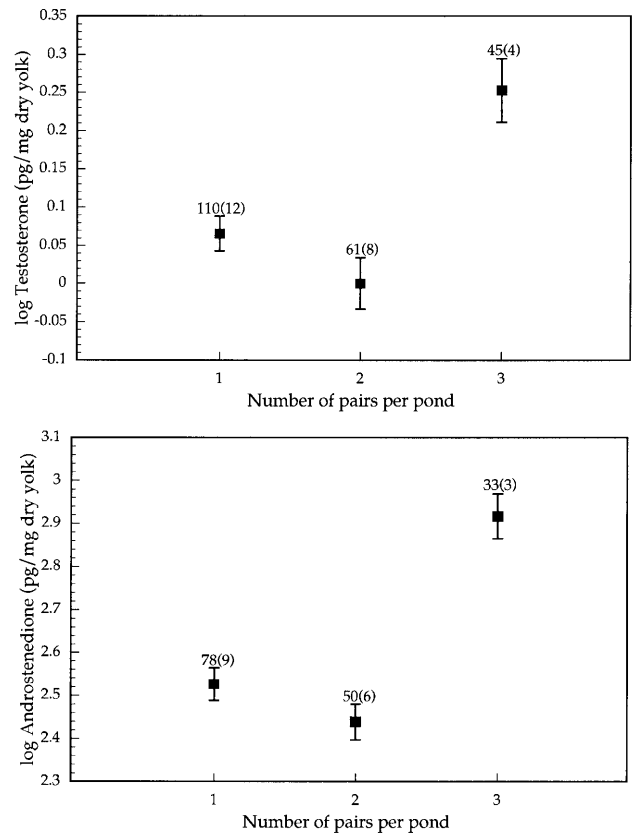


Fig. 6 Mean testosterone and androstenedione levels in egg yolks from ponds with either one, two, or three nesting pairs. Data are log-transformed and presented as means and SEs with the number of eggs in each category and the number of nests in parentheses above the SE bars

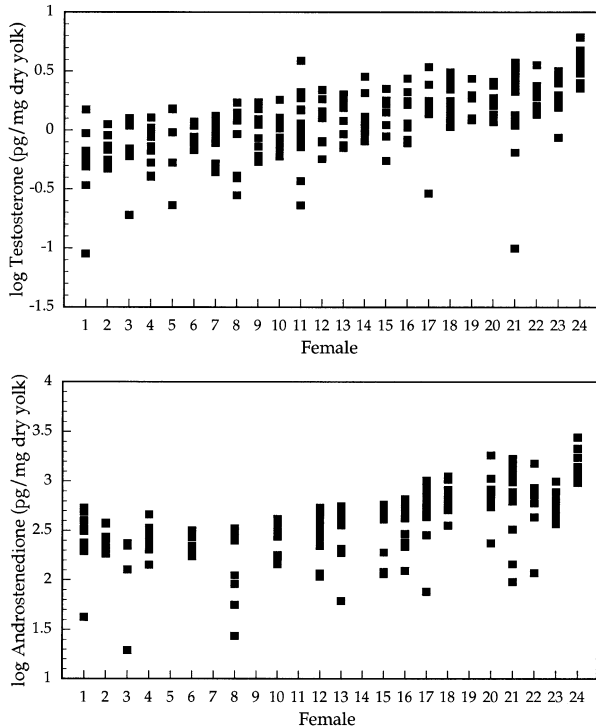


Fig. 5 Variation in testosterone and androstenedione levels in egg yolks produced by each female. Data are log-transformed and each point represents the androgen level for an egg. Female identification numbers were assigned to females by ranking them according to mean testosterone levels in their eggs

males (Fig. 5). There was a statistically significant effect of females, and the number of pairs nesting on the pond from which the clutch was sampled, on both hormones (testosterone: female effect $F_{21,216}=7.10$, $P<0.0001$, pairs/pond effect $F_{2,215}=21$, $P<0.0001$, androstenedione: female effect $F_{15,160}=5.57$, $P<0.0001$, pairs/pond effect $F_{2,160}=38$, $P<0.0001$). Testosterone and androstenedione levels in egg yolks were significantly higher for females on ponds with three nesting pairs than on ponds with only one or two nesting pairs (Tukey-Kramer HSD $q_{215,3}=2.36$, $P<0.05$, Fig. 6).

Discussion

We found no variation in testosterone levels within individual American coot eggs, but there was a distinct pattern of variation in androgen levels among eggs within clutches. Most of the variation was among eggs from different females, and this was partly accounted for by the number of pairs nesting on the pond.

Within-egg variation

One criticism of yolk biopsies from eggs is that systematic variation within yolks may mask or bias patterns of variation among eggs if biopsies are not consistently taken from the same yolk layer in all eggs. Variation in hormone levels within individual eggs could exist as a by-product of variation in ovarian steroidogenesis during yolk deposition. Lipar et al. (1999b) found that the external layers of yolks for both red-winged blackbirds (*Agelaius phoeniceus*) and dark-eyed juncos (*Junco hyemalis*) contain lower yolk testosterone levels than the intermediate or interior yolk layers. This pattern is consistent with changes in follicular levels of testosterone during yolk deposition measured in domestic fowl (*Gallus gallus domesticus*, Bahr et al. 1983). Whatever the cause of these gradients, variation at this level could influence the timing of exposure to androgens during embryo development if yolk is used by the embryo according to the layers. Coot eggs show no such gradient. If yolk hormone levels reflect follicular steroidogenesis prior to ovulation, our data suggest that follicular androgen production is constant during yolk deposition in coots or that steroids diffuse uniformly throughout the yolk within a short time. Systematic variation in androgen levels within eggs appears to be species-dependent, and for coot eggs, yolk biopsies in the field provide an appropriate way to sample maternal hormone deposition.

Within-clutch variation

Within clutches, coot egg yolks vary in testosterone and androstenedione levels such that the last-laid eggs have lower concentrations than eggs laid earlier in the sequence. Testosterone levels increase with laying sequence in canaries (Schwabl 1993), dark-eyed juncos, and red-winged blackbirds (Lipar et al. 1999a, 1999b), whereas in zebra finch (*Taeniopygia guttata*) androgen levels decrease with laying sequence (Gil et al. 1999), and in cattle egrets (*Bubulcus ibis*) the last-laid egg of a three-egg clutch has the lowest yolk testosterone level (Schwabl et al. 1997). In the American kestrel (*Falco sparverius*) only the first egg laid by a female has lower androgen levels than the remaining eggs in the clutch (Sockman and Schwabl 2000). In several cases these patterns of variation in yolk androgen levels have been implicated in mediating sibling interactions after hatching. Schwabl (1993) found that sibling canaries from eggs with high levels of testosterone had a higher social dominance rank than siblings from eggs with low testosterone levels. Schwabl (1993) and Lipar et al. (1999a) further suggested that, through these behavioral effects, the higher testosterone levels of later-laid eggs mitigate effects of size hierarchies established via hatching asynchrony. In cattle egrets the effect of hatching asynchrony on sibling interactions appears to be exaggerated by androgen levels in the yolk, because the first-laid (and first-hatched) young come from eggs with higher levels

of yolk testosterone than young from the last-laid egg (Schwabl et al. 1997). Sibling aggression is high in cattle egrets and often results in siblicide, with the last-hatched young usually suffering mortality (Ploger and Mock 1986). On the other hand, elevated androgen levels in first-laid eggs may provide later-laid eggs with an advantage. Sockman and Schwabl (2000) recently reported that artificially elevating androgens in the first-laid egg of American kestrels prolonged its incubation period and reduced chick growth rate and survival. It is not clear from this study, however, why later-laid eggs with the same androgen levels as eggs with artificially elevated testosterone did not suffer the same consequences.

The pattern of intra-clutch variation in coots may exaggerate effects of hatching asynchrony as has been reported in cattle egret clutches (Ploger and Mock 1986) if yolk androgens heighten aggressive behavior in chicks. Clutch sizes in coots range from 5 to 16 eggs, and females begin incubation after the third to sixth egg is laid (Alisauskas and Arnold 1994). The first three to six eggs usually hatch nearly synchronously, and the remainder hatch at the rate of one egg per day thereafter. In some clutches there can be as much as an 8- to 10-day age difference between the first and last chicks to hatch. Our results indicate that the first chicks to hatch come from eggs with higher levels of testosterone and androstenedione than their later-hatched siblings, and if androgen levels in eggs are beneficial, this effect could exacerbate any effects of hatching asynchrony on survival of later-hatched young.

Another maternal investment that varies with laying sequence in coots is egg size. Coot eggs vary in size with laying sequence in a similar pattern to that of testosterone and androstenedione levels (Arnold 1991; Reed 2000). The first-laid egg is small, the second to fourth eggs are largest and then egg size decreases with laying sequence, and on average there is a 2-g (~7% of egg mass) difference between the largest egg and the smallest egg in a clutch (Reed 2000). The difference in androgen levels among eggs within clutches averages 1.1 pg mg⁻¹ dry yolk for testosterone and 550 pg mg⁻¹ dry yolk for androstenedione (Fig. 4). In a previous study we determined that the relative differences in size among eggs within clutches are associated with differences in survival of young, independent of effects of hatching asynchrony (Reed 2000). We used a fostering design (controlling for clutch size, post-hatching parental care, and hatching asynchrony) and found that young hatching from the largest eggs in a clutch had higher survival than chicks from the smallest eggs in a clutch. We suggest that this survival difference is likely due to maternally derived resources present in the egg (Reed 2000). Androgen levels and egg size show a similar pattern with laying sequence; the largest eggs within the clutch also have the highest concentrations of yolk androgens. Although we do not know the mechanism involved, the relatively high levels of androgens and large egg sizes may enhance the survival probability of young from early-laid eggs in a clutch. The exception is the

first-laid egg in the clutch which is both smaller and has lower yolk androgen levels than subsequent eggs. Concordance of these size and hormone patterns suggests that absolutely more resources, such as yolk, albumen and hormones are available to earlier-hatching offspring and fewer of these resources are available to later-hatching offspring. In coots clutch sizes are large and hatching asynchrony is extreme, and unequal allocation of resources among offspring may be the optimal solution for females distributing a limited number of resources among many offspring.

Among-clutch variation

Most of the variation in yolk androgen levels in American coots is due to differences among eggs from different females. This variation may be sensitive to environmental stimuli that affect circulating levels of hormones in the laying females and thus hormone deposition in eggs. This could provide a mechanism for a female to transmit information about current environmental conditions to her offspring and subsequently influence their behavior, morphology, or physiology (e.g., Shine and Downes 1999).

There are several examples in which variation in females' environments prior to egg production are reflected in offspring traits. In a free-living population of tropical damselfish (*Pomacentrus amboinensis*), cortisol, a stress hormone, is influenced by the level of inter- and intra-specific interactions. Increased social interactions result in high levels of maternal and egg cortisol levels and decreased larval size at hatching (McCormick 1998). In this case, maternally derived hormone resources in eggs have a negative effect on offspring performance. In a population of black-headed gulls (*Larus ridibundus*), females nesting in high-visibility sites, in which the number of social interactions among conspecifics is high (Bukacinska and Bukacinski 1993), lay eggs with higher yolk androgen levels than eggs laid by females nesting in low-visibility sites (Eising et al. 2001). Black-headed gull chicks exhibit testosterone-mediated aggression while defending territories against conspecifics (Groothuis and Meeuwissen 1992) and elevated levels of androgens in eggs may be advantageous for the chicks hatching in these environments (Eising et al. 2001). Female zebra finch (*Taeniopygia guttata*) respond to preferred morphological characters of their male partners by increasing androgen levels in eggs fathered by these males (Gil et al. 1999). Female house sparrows (*Passer domesticus*) nesting in colonies lay eggs with higher testosterone levels than females nesting singly (Schwabl 1997). In each of these cases, allocation of hormones to eggs is a phenotypic trait that exhibits plasticity and is sensitive to social context and cues experienced by the mother prior to egg production.

In American coots, inter-individual variation in yolk androgens also appears to be sensitive to level of conspecific interactions. Females nesting on ponds with two

other coot pairs lay eggs with higher androgen levels than females nesting either singly, or nesting on ponds with only one other pair. Male and female coots vigorously defend their breeding territories from conspecifics, but rarely leave their pond during the nesting season to interact with other birds (Gullion 1953). The greater the number of pairs nesting on a pond, the more likely that adults will engage in aggressive encounters during territory defense (authors, personal observations). The effect of such aggressive encounters on circulating levels of androgens is well documented in male birds (e.g., Wingfield et al. 1987, 1994) and to a lesser extent in females (Groothuis and Meeuwissen 1992; Sandell and Smith 1997; Staub and De Beer 1997). Our results support the hypothesis that the territory defense behavior of an adult female, prior to egg laying, elevates androgen levels and consequently affects the levels of androgens deposited in eggs.

Inter-individual variation in androgen levels may also reflect genetic differences in androgen production among females. In a system in which females exhibit natal philopatry, related females (sisters, or mothers and daughters) might tend to nest on the same pond. In addition, if genes contributed to the variation in androgen production among females (e.g., Maxson et al. 1983; Künzli and Sachser 1999) there would be a tendency for yolk androgen levels of females within a pond to be more similar than yolk androgen levels in females among ponds. Thus, the spatial association of relatives could contribute to the observed patterns of inter-individual variation. Information about the relative contribution of environment and genes to existing inter-individual variation is needed to understand the evolutionary and functional significance of variation among individuals.

Interpretation of variation in yolk androgens

Androgens in avian egg yolks have been assumed to provide a selective advantage for offspring growth and survival (Winkler 1993; Schwabl 1993, 1996; Schwabl et al. 1997; Gil et al. 1999; Lipar et al. 1999a; but see Sockman and Schwabl 2000). The empirical evidence for this effect, however, is limited to two studies on domestic canaries: a correlational study between yolk androgens and social dominance later in life (Schwabl 1993) and a manipulative experiment in which increases in growth and begging behaviors were observed in chicks from testosterone-treated eggs (Schwabl 1996). The effects of androgen levels on begging behavior, growth, and social dominance in young are all characters that have utility in mediating sibling interactions. Thus, we might expect to find that the pattern of variation in yolk androgens affects survival within clutches. However, the greatest differences in yolk androgens are among females (Lipar 1999a; this study) and the adaptive significance of among-female variation in yolk androgen levels is not clear. If there are positive effects of absolute levels of yolk androgens on offspring performance, we

would expect to see a concordance between among-female variation in yolk androgens and offspring performance among clutches. Currently we have no evidence to suggest that this is the case. The fact that yolk androgen levels vary so greatly among females, along with the possible beneficial effects of yolk androgens on offspring, raises the question of why do all females not enhance their offspring's performance by investing more androgens in their eggs.

As with many other traits, there are likely trade-offs that constrain the evolution of ever-increasing yolk androgens (e.g., Stearns 1992). Hormones have widespread and multiple effects on phenotypes, and can simultaneously affect an animal's behavior, growth, morphology, and development. Thus, any two traits, or suite of traits, that are affected by a single hormone are mechanistically linked in a similar sense to pleiotropic effects of genes that link traits (Ketterson and Nolan 1999). Maternal effects are additionally complex because the traits are shared between the female and her offspring. Trade-offs may occur between offspring, benefiting from high yolk androgens, and females, suffering from high levels of circulating androgens necessary for the deposition of those androgens. Negative effects of high androgen levels could include decreased parenting or incubation behaviors (Silverin 1980; Hegner and Wingfield 1987; Oring et al. 1989; Ketterson et al. 1992; Vleck and Dobrott 1993; Saino and Møller 1995), decreased immune function (Folstad and Karter 1992; Saino et al. 1995; Hillgarth et al. 1996; Salvador et al. 1996; Zuk 1996), or suppression of follicular development (Hillier and Ross 1979). Part of the variation in androgen levels among females is attributable to social interactions and the potentially negative effects on female physiology may be one mechanism underlying density-dependent effects on reproduction. Understanding the adaptive significance of patterns of variation in yolk androgens will require a better understanding of the physiological, environmental and genetic bases for this variation, the contribution of yolk androgens to offspring performance in free-living birds, and the trade-offs that constrain the evolution of maternal androgen investments in eggs. Given the ability to manipulate androgens levels independently in either eggs or females, it should be possible to isolate the consequences of increased (or decreased) androgen levels on offspring and female performance.

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