

Maternal androgens in eggs of communally breeding guira cuckoos (*Guira guira*)

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Abstract

Variation of maternal androgens in avian eggs may be a mechanism of maternal influence on offspring development, growth, and/or behavior. We studied yolk androgen concentrations in eggs of guira cuckoos (*Guira guira*) to understand how females might enhance the success of offspring in a complex communal breeding system. We measured concentrations of androstenedione, 5 α -dihydrotestosterone, and testosterone in yolks and identified eggs and clutches of individuals in joint nests by yolk protein electrophoresis. Androstenedione had the highest yolk concentration, at least 10 times higher than that of testosterone and 5 α -dihydrotestosterone. The first eggs of individual females that laid two or three eggs in a joint nest had lower androstenedione concentrations than their second and third eggs, the latter having a lower probability of being ejected from the nest. This implies that guira cuckoo females may influence offspring survival and competitiveness in communal nests by means of differential allocation of androstenedione and laying tactics. There was significant variation in yolk androstenedione among females, but the order in which females entered laying in the communal clutch had no effect on the concentrations. Androstenedione yolk concentrations increased with communal clutch size, which may indicate that higher levels of competition in larger groups lead to higher yolk androgen concentrations. Finally, androstenedione concentrations were higher in clutches in the later wetter periods of the rainy season than during the earlier drier period. This may be explained by the high frequency of large clutches in the later periods, with more females contributing to a joint clutch. © 2006 Elsevier Inc. All rights reserved.

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Introduction

Parent birds can influence the fitness of their offspring in several ways, favoring or handicapping particular individuals in a brood. For example, they can hatch some eggs before others (hatching asynchrony) by initiating incubation before completion of the clutch (reviewed in Magrath, 1990). This frequently leads to a size hierarchy among nestlings where the earliest hatched young have a competitive advantage over the later hatched young, especially with respect to food access (Ricklefs, 1993; Mock and Parker, 1997; Drummond, 2001). Alternatively, females can vary the amount of nutrients allocated to eggs,

thereby influencing offspring quality. Larger eggs may contain more water, protein, and lipids, which may benefit hatchlings by increasing their growth rate and survival chances (reviewed in Williams, 1994). Variation in steroid hormone concentrations in the eggs, specifically androgens, is an additional mechanism of maternal influence on offspring fitness (Schwabl, 1993, 1996a,b, 1997; Schwabl et al., 1997; Gil et al., 1999; Lipar and Ketterson, 2000; Lipar et al., 1999; Sockman and Schwabl, 2000; Eising et al., 2001; Pilz et al., 2004). Androgen concentrations in avian eggs often vary with laying order and may affect offspring fitness positively or negatively by influencing their development, growth, and/or the ability to compete with siblings for parental resources in the nest. Yolk androgens vary with laying order within females and with yet to be fully understood factors among females, one of them being the social conditions experienced by the mother.

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Despite a host of studies on advantageous effects of yolk androgens for the offspring, it remains unclear whether females can actively adjust yolk androgen concentrations in their eggs or whether yolk androgens are a passive reflection of androgen production for other ends such as egg formation or female behavioral changes. Variation in the concentration of yolk androgens has been shown to be correlated with circulating concentrations of androgens in the female during yolk formation (Schwabl, 1996a, 1997) and has been shown to be influenced by the female's social conditions (Whittingham and Schwabl, 2002; Müller et al., 2002; Pilz and Smith, 2004). For example, more intense levels of competition associated with higher nesting densities could lead to enhanced secretion of androgens.

In this study, we examined androgen concentrations in eggs of the South American guira cuckoo (*Guira guira*), a Crotophaginae species that has a complex and variable social structure. Guira cuckoos are among the few species of birds that reproduce communally by laying in a joint nest and cooperating in the incubation of eggs and rearing of nestlings (Macedo, 1992, 1994; Quinn et al., 1994). Their reproductive groups may have up to 15 related and unrelated individuals (Quinn et al., 1994) and as many as seven females laying simultaneously in a joint nest (Cariello et al., 2002). Communal clutch sizes are positively correlated with the number of reproductive females (Cariello et al., 2002) and may range from one to as many as 26 eggs. Surviving eggs usually hatch synchronously (Macedo, 1992), suggesting that asynchronous incubation is not used as a mechanism to influence sibling competition. Although breeding groups may nest up to five times in the same territory during a single breeding season (Melo, 1997), females do not always contribute to each nesting attempt of their groups nor do they retain the order of entering laying. Some females lay more eggs in communal clutches than do other females (Cariello et al., 2002; Macedo et al., 2004a). There is considerable competition for reproductive opportunities in the group as indicated by egg ejection, which is more prevalent at the onset of laying (Macedo, 1992; Macedo et al., 2004b), and by infanticide, which typically occurs during the first 5 days post-hatch (Macedo and Melo, 1999; Macedo et al., 2001).

We investigated yolk androgen concentrations in eggs of guira cuckoos in a social context. First, we asked whether there is, similar to many other species, a relationship between yolk androgen concentrations and laying order for individual females in communal clutches of the guira cuckoo. Second, considering that increased levels of competition are associated with elevated levels of androgens in eggs in several species, as mentioned above, we examined whether the concentrations of androgens in yolk vary with the size of the communal clutch. As communal clutch size is positively correlated with the number of laying females in the group, this parameter could be considered an indicator of level of competition among females. Data from a previous study support this assumption: the lowest egg ejection probabilities occur in very small communal clutches (1–3 eggs) where there are only one or two laying females (Macedo et al., 2004b). Ejection levels become

higher as more females contribute eggs to the joint clutches and compete for nest space for their eggs. Additionally, eggs laid in smaller communal clutches have less nutrients, on average, than those laid in larger communal clutches. In large communal clutches, where there are offspring of many females, it may be important for eggs to be of higher quality. Larger eggs in this species produce heavier and larger chicks, and chicks victimized by infanticide are lighter and smaller at hatching than those that survive (Macedo et al., 2004b). These results suggest the possibility of reproductive manipulation by females depending upon social condition that may enhance individual fitness. Third, we asked whether the order of entrance of females into the laying sequence of the communal clutch is reflected in yolk androgen levels and whether there are differences between eggs ejected from or retained in the communal clutch. Finally, we tested whether yolk androgens vary with seasonal changes in ecological conditions associated with the progress of the breeding season.

Materials and methods

Study area

The study population of guira cuckoos is located in the Central Brazilian Plateau, in a semi-urban area of 30 km² near Brasília (15°47'S, 47°56'W; altitude 1158 m). This area is characterized by a combination of patches of native "cerrado" vegetation, cultivated fields, and urbanized plots, and the guira cuckoos favor introduced monkey puzzle trees *Araucaria angustifolia* for nesting. There are clearly defined rainy and dry seasons, and reproductive activity is mostly restricted to the rainy season, which ranges from mid-August to mid-March with peak reproduction in our study site usually occurring in September and October.

Field procedures

Details of field procedures are described elsewhere (Cariello et al., 2002). In reference to our field and laboratory-related activities, IBAMA/Brazil authorized the collection of eggs (076/98-DIFAS and 027/99-DIFAS permits), and all procedures complied with Brazilian and USA current laws. In brief, from August to December 1998 and 1999, we visited active nests daily to record breeding events and to collect freshly laid eggs, which we substituted with dummy eggs in the nest. The dummy eggs were numbered sequentially as they were used in the substitution process to record the egg ejection patterns. We placed fishing nets below nests for collection of eggs being ejected from nests. Ejection patterns of eggs were not different when comparing years with or without egg substitution (Macedo et al., 2004a). Fresh eggs were broken apart, and the yolk and egg white weighed separately. Yolks were frozen at –20°C.

Maternal identification of eggs

We conducted electrophoretic analyses of yolk proteins to identify maternity of eggs of individual females in communal nests of guira cuckoos as detailed in Cariello et al. (2002). Concisely, yolk samples were homogenized with distilled water, mixed with 67 mM potassium phosphate buffer and trypsin (for protein digestion), and then incubated for 2 h at room temperature. The digestions were stopped after incubation by heating to 94°C for 30 s. Samples were run on 12.0% acrylamide separating gels under denaturing conditions according to Laemmli's (1970) protocol for 2 h at 20 mA and 80 V at room temperature. Silver staining was used for visualizing protein fragments on gels (Morrissey, 1981). Through this electrophoretic technique, we found that most guira cuckoo females contributing two or more eggs to the communal clutch (74%) laid every 2 days, but laying intervals of 1 (16%) and 3 days (10%) sometimes occurred.

Measurement of yolk androgens

We followed the standard protocol (Schwabl, 1993) for the extraction, separation, partial purification, and measurement by radioimmunoassay (RIA) of yolk androstenedione (A_4), 5 α -dihydrotestosterone (DHT), and testosterone (T) concentrations. We homogenized thawed yolks with distilled water (1:1) and transferred weighed aliquots of the homogenate (mean mass of yolk used in assays: 159 mg for A_4 and T and 199 mg for DHT) to extraction tubes. We then added approximately 2000 cpm of each tritiated steroid to each homogenate to measure extraction and purification recoveries. We extracted steroids twice with 4 ml petroleum and diethyl ethers (30:70) and precipitated lipids and proteins in 90% ethanol at -20°C . We then partially purified and separated steroids on diatomaceous earth microcolumns before measuring androgen levels. The A_4 fraction was collected with 2% ethyl acetate in isooctane, the DHT fraction with 10% ethyl acetate in isooctane, and the T fraction with 20% ethyl acetate in isooctane. The sensitivity of the standard curve for A_4 was 3.9 pg/tube and for both DHT and T was 1.95 pg/tube each. The sensitivities calculated for a mean yolk mass of 159 mg for A_4 and T and 199 mg for DHT were 0.161, 0.084, and 0.117 pg/mg yolk, respectively. We measured yolk A_4 and T in seven assays and DHT in six assays. The average A_4 , DHT, and T intra-assay coefficients of variation were 12.3, 10.9, and 19.6, respectively. The inter-assay coefficients of variation were 23.3%, 20.1%, and 34.4% for A_4 , DHT, and T, respectively. All eggs in a communal clutch were analyzed in the same assay.

Statistical analyses

Concentrations of A_4 , DHT, and T were not normally distributed (they were strongly left skewed), therefore we log transformed A_4 and DHT and used the reciprocal of T, after which the data met assumptions of normality (Zar, 1999). We used repeated measure ANOVA to analyze variation of androgen concentrations in eggs of individual females in communal clutches. Since hormone concentrations differed between years for A_4 (t test, $t_{1,193} = 5.12$, $P < 0.001$), DHT (t test, $t_{1,193} = -3.86$, $P < 0.001$), and T (t test, $t_{1,193} = -12.37$, $P < 0.001$), we used randomized block ANOVA (RB ANOVA) for comparisons of androgen concentrations and effect variables tested (clutch size, order of female entrance in laying sequence, and season), removing the effect of the year. Bonferroni's adjustments for multiple comparisons were used for hormone data as a method guaranteed to be conservative. Significance level for all tests was set at $P \leq 0.05$.

Results

We monitored 297 eggs from 48 communal guira cuckoo nests in 1998 and 1999. From this total number, 195 fresh eggs laid by 82 females in 34 nests were available for RIA analyses and maternal identification. Eggs not available for the analyses included 59 eggs found during incubation, which could not be used as both electrophoretic and hormone analyses require fresh laid eggs, and 43 eggs were lost due to ejection to the ground.

There was high variability in concentrations of all androgens measured in guira cuckoo eggs, and the range of variation was greater in 1998 than in 1999; the steroid with the highest concentration was A_4 in both years (Table 1). Average DHT and in particular T concentrations were less than 1 pg/mg, much lower than those reported for most other species. In some samples, T fell below the detection limit. Non-detectable values were set at the detection limit as a conservative estimate for statistical analyses. All three androgens were positively correlated with one another, although the amount of variability in one androgen accounted for by the variability of the second androgen was low in all cases, as confirmed by the values of r^2 (A_4 —DHT: $r^2 = 0.028$, $P = 0.019$; A_4 —T: $r^2 = 0.185$, $P < 0.001$; DHT—T: $r^2 = 0.201$, $P = 0.005$; sample of 195 eggs for all correlations).

Table 1

Mean hormonal concentration in guira cuckoo eggs during two study years

Androgen	Hormone concentrations (pg/mg yolk)			
	1998		1999	
	Mean \pm SEM (n)	Range	Mean \pm SEM (n)	Range
A_4	11.809 \pm 1.353 (113)	0.870–93.640	6.227 \pm 0.791 (82)	0.190–34.590
DHT	0.484 \pm 0.056 (113)	0.090–3.150	0.520 \pm 0.033 (82)	0.160–2.090
T	0.419 \pm 0.070 (113)	0.080–4.490	0.107 \pm 0.013 (82)	0.040–0.830

Yolk androgen in individual female clutches

We were able to determine maternity of all eggs in 16 communal nests (that is, we collected all retained eggs, and all ejected eggs were caught by the nets). Therefore, we used data from these groups to investigate variation of androgen concentrations within individual female clutches in guira cuckoo nests. In these groups, 39 females laid from one to three eggs, and, for comparative purposes, we included in these analyses only females laying two or three eggs.

Levels of A_4 varied significantly with laying order within individual clutches ($F_{2,54} = 8.78$, $P < 0.001$). The first egg in each individual clutch had lower A_4 concentrations than second or third eggs. Second and third eggs did not differ in A_4 yolk levels (Fig. 1A). Concentrations of yolk DHT (Fig. 1B) and T (Fig. 1C) showed no intra-clutch variation associated with individual laying sequence ($F_{2,54} = 0.11$, $P = 0.89$; and $F_{2,54} = 0.93$, $P = 0.40$, respectively).

Females differed in the mean concentrations of A_4 (RB ANOVA, $F_{1,80} = 2.41$, $P < 0.001$), DHT (RB ANOVA, $F_{1,80} = 5.08$, $P < 0.001$) and T (RB ANOVA, $F_{1,80} = 2.07$, $P < 0.001$) in their eggs. However, within nests where at least two females contributed eggs to the communal clutch, females only differed from their nestmates with respect to mean yolk A_4 (nested ANOVA with female within nest, $F_{23,64} = 1.69$, $P = 0.01$, $n = 65$ females), but not in mean DHT and T levels (nested ANOVA with female within nest, $F_{23,64} = 1.38$, $P = 0.08$; and $F_{23,64} = 1.01$, $P = 0.48$, respectively).

We tested whether the order of entrance in the laying sequence of the communal clutch could be a source of difference in mean A_4 levels among females. To maximize contrasts for this analysis, we compared yolk A_4 levels of eggs laid by females entering first in the laying sequence (first females) with eggs laid by females entering last in the laying sequence (last females). There was no difference in A_4 concentrations between first and last females (RB ANOVA, $F_{2,38} = 0.00$, $P = 0.99$; mean \pm SEM of 3.82 ± 1.30 and 3.92 ± 1.31 pg hormone/mg yolk for first ($n = 21$) and last females ($n = 18$), respectively).

At least 13 females contributed eggs to two nesting attempts of the same group, and at least one female participated in three renestings of the same group. For those participating in two nesting bouts, we found that their mean A_4 concentration in eggs differed between renestings (t test, $t_{1,12} = -2.73$, $P = 0.02$).

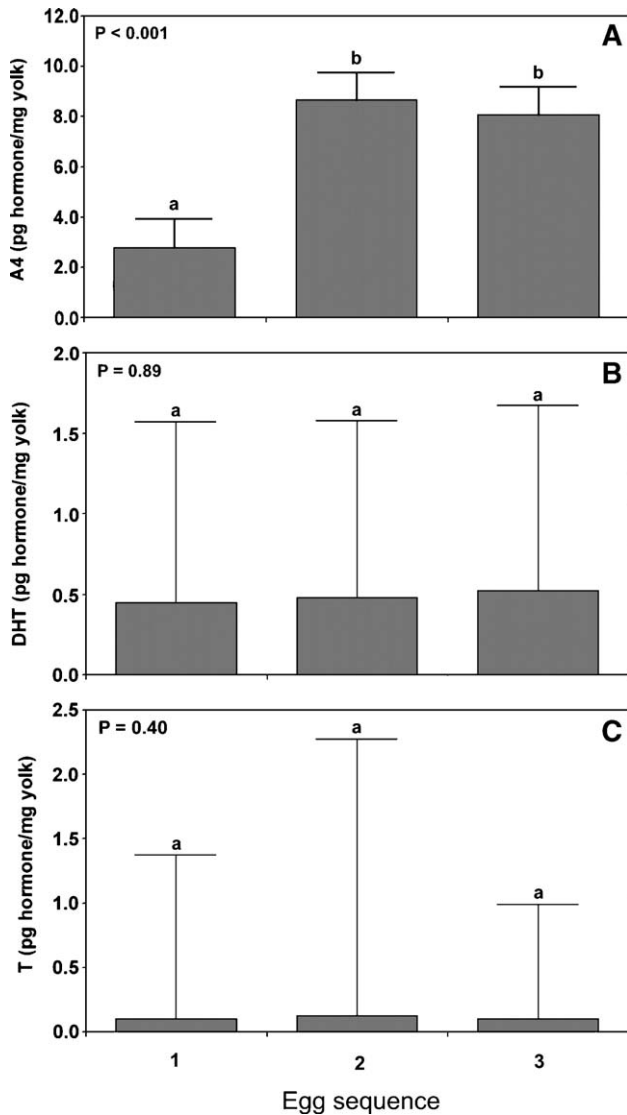


Fig. 1. Mean concentrations (\pm SEM) of androgens. (A) androstenedione (A_4); (B) dihydrotestosterone (DHT); and (C) testosterone (T) in egg yolks as a function of laying order within individual clutches of guira cuckoo females. Different letters above bars indicate significant differences at $P < 0.05$.

On average, eggs laid by individual females in the first nesting bout had a lower A_4 concentration than eggs they laid in the subsequent nesting attempt (mean \pm SEM of 1.89 ± 1.39 and 4.65 ± 1.18 pg hormone/mg yolk for first and second nesting bouts, respectively). For DHT and T levels, such differences were not significant (t test, $t_{1,12} = -1.60$, $P = 0.14$, and $t_{1,12} = -0.12$, $P = 0.91$, respectively).

Yolk androgen in social and seasonal contexts

In communal nests of guira cuckoos, first laid eggs have the highest probability of being ejected. The first egg laid in joint nests had lower A_4 concentrations than the last egg (RB ANOVA, $F_{1,39} = 18.96$, $P < 0.001$). However, there was no difference for DHT and T levels (RB ANOVA, $F_{1,39} = 0.27$, $P = 0.61$ and $F_{1,39} = 2.68$, $P = 0.11$, respectively).

We also compared androgen levels of ejected versus retained eggs, independent of their positions in the laying sequence. To different extents, egg ejection affects all females, independent of their order of entering the laying sequence. We found that there was no significant difference between the levels of A_4 (RB ANOVA $F_{1,153} = 0.59$, $P = 0.45$), DHT (RB ANOVA $F_{1,153} = 0.03$, $P = 0.86$) and T (RB ANOVA $F_{1,153} = 1.27$, $P = 0.26$) for tossed and retained eggs.

To investigate whether hormone levels varied in communal clutches of different sizes, we divided them into three categories: small (1–4 eggs), medium (5–7 eggs), and large (>7 eggs). Communal clutch size is positively correlated with the number of laying females in communal nests of guira cuckoos ($r^2 = 0.90$, $P < 0.001$, $n = 16$ nests: Cariello et al., 2002). This may be considered an indirect indicator of competition among both hatchlings and females and thus, possibly, aggressiveness among females. As communal clutch size increased, A_4 levels increased (RB ANOVA, $F_{2,194} = 5.57$, $P < 0.01$). Large communal clutches had eggs with significantly higher levels of yolk A_4 than small clutches. Small and medium communal clutches did not differ in A_4 levels (Fig. 2). For DHT and T, however, there was no variation associated with

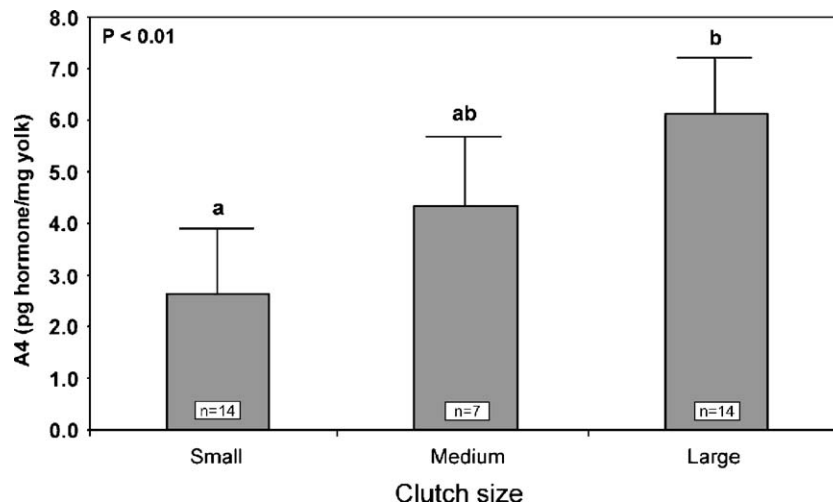


Fig. 2. Mean concentrations (\pm SEM) of androstenedione (A_4) in guira cuckoo egg yolks in small (1–4 eggs), medium (5–7 eggs), and large (>7 eggs) communal clutches (n = number of nests). Different letters above bars indicate significant differences at $P < 0.05$.

Table 2
Climatic and habitat characteristics of the seasonal periods of reproduction of guira cuckoos in the study area

Seasonal period	Interval	Climate characteristics/ Precipitation (mm) ^a	Insect abundance estimates ^b
1	1st Aug–15th Sep	Relatively dry/17.2	Peak abundance for Homoptera and Lepidoptera
2	16th Sep–31st Oct	Beginning of the rains/155.9	Peak abundance for Homoptera and Lepidoptera
3	1st Nov–15th Dec	Intensive rains/341.1	Peak abundance for Coleoptera, Hymenoptera, Isoptera, and Orthoptera

^a Average precipitation in the study area region from 1995 to 1999. Data were registered at Reserva Ecológica do IBGE (15°57'S; 47°53'W, altitude 1100 m). See www.recor.org.br for details.

^b Rough estimates for peak abundances of insects within the time period of the present study and disregarding other possible peaks during the rest of the year, based on Pinheiro et al. (2002).

communal clutch size (RB ANOVA, $F_{2,194} = 2.21$, $P = 0.11$; $F_{2,194} = 0.63$, $P = 0.53$, respectively).

To investigate whether clutch size and hormone concentrations had a temporal component, we divided the reproductive season into three periods of 1.5 months each, which had different characteristics relative to amount of rain and abundance of food resources for guira cuckoos, which are predominantly insectivorous (Table 2). The amount of precipitation increases steadily throughout the guira cuckoo reproductive season. Dur-

ing the study period (which does not encompass the whole rainy season), peak abundances of insects in four orders occurred in seasonal period 3 of the study, as compared to peak abundances in two orders during the first two drier periods. A higher number of large communal clutches occurred during seasonal period 2 (beginning of rains), in comparison to the other two periods (Kruskal–Wallis, $H = 9.56$, $P = 0.008$, Fig. 3A). Highest concentrations of A_4 also occurred during this part of the reproductive season (Fig. 3B). Clutches laid during periods 2 and 3 of the season (beginning of rains and intensive rains, respectively) had significantly higher levels of A_4 than those laid during period 1 (relatively dry) of the reproductive season (RB ANOVA, $F_{2,194} = 4.60$, $P = 0.01$). Eggs laid at the beginning of the rains (period 2) and during the subsequent period of intensive rains (period 3) did not differ regarding A_4 yolk concentrations. DHT and T levels did not vary seasonally (RB ANOVA, $F_{2,194} = 1.02$, $P = 0.36$ and $F_{2,194} = 1.92$, $P = 0.15$, respectively).

Discussion

The major steroid in guira cuckoo egg yolk was A_4 , but its concentrations were low compared to other species. DHT and in particular T concentrations were even lower, and low compared to other altricial species. Low DHT and T concentrations and elevated A_4 concentrations are characteristic of non-passerine species (Schwabl et al., personal communication). The

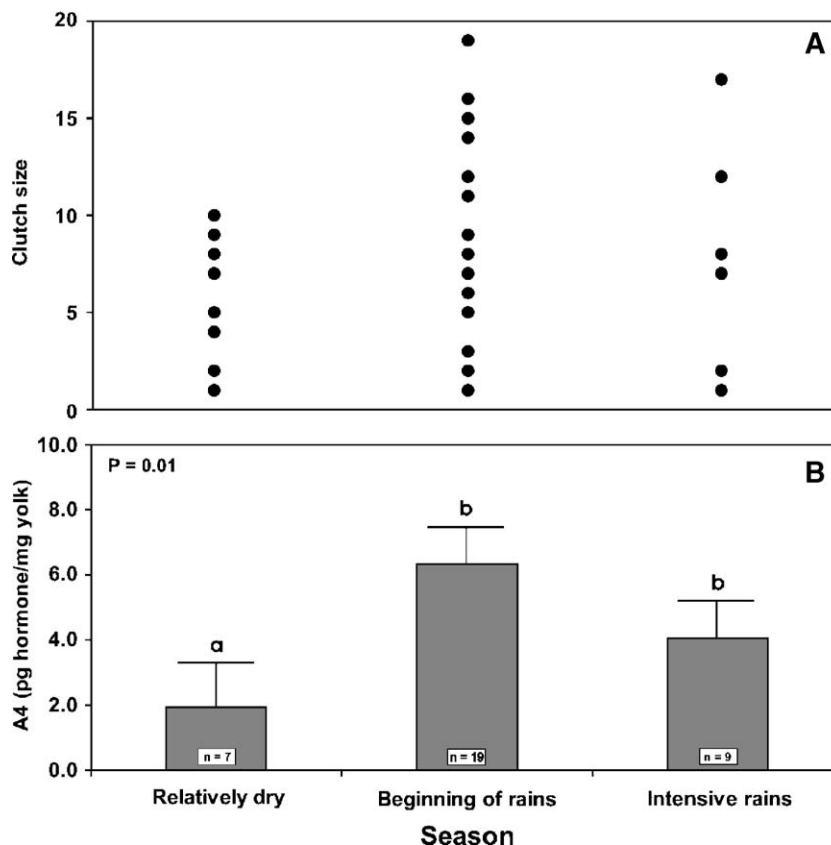


Fig. 3. (A) Size of guira cuckoo communal clutches laid in different periods of the reproductive season; and (B) mean concentrations (\pm SEM) of yolk A_4 in eggs laid in different periods of the reproductive season (n = number of nests). Different letters above bars indicate significant differences at $P < 0.05$.

guira cuckoo shows, just like passerine birds, an altricial development mode. In passerines, A_4 is low and T and DHT are high and negatively correlated with embryo and nestling development (Schwabl et al., personal communication). Thus, it seems that phylogeny, in particular, the evolution of the passerines, influenced the concentrations of androgens into yolks and that the altricial development mode is not necessarily associated with elevated T and DHT yolk concentrations.

Latitude is another factor that has to be considered in studies of tropical birds as it may be a very influential factor. Plasma T levels of male tropical birds exhibit lower mean peak values than those of northern temperate birds (Goymann et al., 2004), which suggests that breeding season length is the major determinant of T levels in tropical birds: the shorter the breeding season, the higher the testosterone level. This latitude influence is consistent with our results of low T levels in guira cuckoo egg yolks as breeding activities in this species may last for over 6 months (Macedo, 1992). Goymann et al. (2004) also found that tropical birds that defend a breeding season territory had higher T levels than birds that were year-round territorial or colonial. However, we must be cautious in interpreting our results as there are no available data on plasma androgens of guira cuckoo males and, consequently, hormone concentrations in males cannot be compared to those in females.

Comparisons in androgen plasma concentrations have also been made between males and females of another tropical bird, the African black coucal *Centropus grilli*, an altricial cuckoo species with a polyandric mating system where males provide all parental care (Goymann and Wingfield, 2004). Despite their atrophied left testis, black coucal males had significantly higher levels of plasma T than did females. However, the authors emphasized that this does not necessarily exclude a role of T in females. As in males, females had significantly higher levels of this steroid during the mating than during the pre-mating period, suggesting that this steroid may influence the regulation of territorial behavior. Goymann and Wingfield (2004) also found that concentrations of A_4 in black coucals were lower than T, which is the opposite of what we found for the guira cuckoos, where A_4 levels were higher than T levels in the egg yolks.

Hau et al. (2004) studied territorial aggression and hormones in the tropical year-round territorial spotted antbird *Hylophylax n. naevioides* during the non-breeding season. Free-living males and females displayed robust and qualitatively similar aggressive behavior towards simulated intruders. However, opponents of the same sex resulted in stronger and more intensive responses of aggressive behaviors by both males and females. In both sexes, plasma dihydroepiandrosterone (DHEA) levels were detectable and higher than T and 17β -estradiol (E2). In males, plasma DHEA levels were positively correlated to simulated intrusions. This finding supports earlier suggestions that this steroid might function as a precursor for active steroids such as T and E2 in both sexes. The possible role of androgen precursors such as DHEA outside the reproductive season might ensure the benefits of T, such as territorial defense, without the possible costs of elevated plasma T on other traits (Wingfield et al., 2001).

Most studies on yolk hormones in birds have suggested that an increase or decrease in androgen levels with laying order might be associated to hatching asynchrony to benefit or handicap selective offspring, respectively (Schwabl, 1993, 1997; Lipar and Ketterson, 2000; Lipar et al., 1999; Eising et al., 2001). In laboratory canaries (*Serinus canaria*), the increased T concentrations in eggs within a clutch resulted in more food begging and faster growth (Schwabl, 1996b), while, in red-winged blackbirds (*Agelaius phoeniceus*), it enhanced the development of the hatching muscle (Lipar and Ketterson, 2000). Elevated yolk androgen levels also enhanced chick growth and food begging in black-headed gulls (*Larus fuscus*; Eising et al., 2001; Eising et al., 2003). In European starlings (*Sturnus vulgaris*), yolk T enhanced growth trajectories in periods of food shortage but without observable effects on begging (Pilz et al., 2004). However, elevated yolk androgen levels may not always be beneficial to chicks. For example, in American kestrels (*Falco sparverius*), combined T and A_4 injections into eggs resulted in delayed hatching and reduced nestling growth and survival compared to control chicks (Sockman and Schwabl, 2000).

In guira cuckoos, the concentrations of all yolk hormones measured, especially those of A_4 , the most abundant androgen, varied over a wide range. One source of A_4 variation was the order in which eggs were laid by females within their individual clutches in joint nests. In this case, A_4 concentrations in first eggs were lower than those in second and third eggs. Although most guira cuckoo eggs hatch synchronously in communal nests, females may be influencing chick survival and competitiveness through differential deposition of androgens within their individual clutches. Up to seven females may lay eggs in guira cuckoo joint nests (Cariello et al., 2002), where full-siblings, half-siblings, and none-siblings (Quinn et al., 1994) compete for food and space. Thus, a guira cuckoo female tactic to raise a successful offspring could be to provide extra doses of androgens to eggs laid later in the clutch, with higher survival probabilities, thus favoring chick competitive abilities (e.g. aggressive behavior and intensive begging), which in turn could result in faster growth and development. However, beyond establishing this variation in A_4 in the laying sequence of individual female clutches, it will be necessary, in future studies, to show whether there is a positive or negative relationship between maternally derived androgens in guira cuckoo eggs and the competitive abilities, growth, and survival rates of nestlings.

The first laid eggs in communal nests of guira cuckoos are disproportionately more likely to suffer ejection, independent of communal clutch size (Macedo, 1992; Macedo et al., 2004b). An egg laid first has about 50% chance of being ejected, significantly more than the 32% chance of ejection for the last egg laid in the communal clutch (Macedo et al., 2004b). It has been found that first-laying females lay eggs with significantly less mass, volume, and yolk than do last-laying females (Macedo et al., 2004b). Another possible explanation for the lower A_4 concentrations found in the first eggs of individual female clutches is that this may be an additional way of minimizing investments in face of the elevated

probability of egg ejection in the beginning of the laying sequence. However, at present, there are no available data concerning the patterns and function of androgen secretion during egg production in birds, as was pointed out by Williams (2005). This author, however, also speculates that, for steroids, there may be trade-offs during reproduction between the female requirements for hormonal regulation and effects upon offspring via maternal transfer to the egg yolk. Further studies are necessary to corroborate or refute this androgen-cost conjecture.

Finally, the individual within-clutch variation in A_4 levels could be a female tactic to destroy eggs being simultaneously laid by co-breeders that have higher androgen concentrations. By laying a first egg with a low androgen concentration, a female would be sacrificing a low investment egg to possible ejection, while simultaneously ejecting other females' eggs, of higher androgen content. Thus, a female could eliminate future competitors of her own chicks at a relatively low cost. In the joint-nesting acorn woodpecker *Melanerpes formicivorus*, females' first eggs are usually runts, and Koenig et al. (1995) suggested that this could be a tactic that allows the female that lays a first runt egg to eject more normal eggs laid simultaneously by other females.

Besides the adaptive explanations proposed above, we cannot discard the possibility that A_4 synthesis by the female is lower early in the laying period and that it increases over laying of subsequent eggs as a necessary by-product of physiological processes during egg production, as has also been suggested to explain the intra-clutch variation of androgen levels in American kestrels (Sockman and Schwabl, 2000). Available information on guira cuckoo physiology, however, does not exist, and further research is needed to test this hypothesis. An important question is how guira cuckoo females are able to direct (whether actively or passively) more androgens to selective eggs. All the plausible explanations suggested above to explain differential concentrations of A_4 within female clutches may not be mutually exclusive. Instead, they may be complementary mechanisms operating simultaneously, through which females influence offspring success.

Additionally to intra-clutch variation of A_4 , guira cuckoo females sharing a nest differed from co-breeders with respect to A_4 concentrations in their eggs. However, the order of entrance of females in the laying sequence was not responsible for this variation. This may result from an apparent lack of consistent dominance among jointly nesting guira cuckoo females (Macedo et al., 2004a). Most females change their position in the laying sequence in repeated nesting bouts of the group and do not contribute eggs to all nesting bouts of their groups (Cariello et al., 2002; Macedo et al., 2004a). Furthermore, independent of the order in which they enter the laying sequence in the nest, all females, even the last-laying one, are usually affected by egg ejection.

In a social context, we found that A_4 levels in guira cuckoo eggs increased with communal clutch size. Since communal clutch size is positively correlated with number of laying females (Cariello et al., 2002), we suggest that higher levels of A_4 in eggs result from more intense competition in larger

groups. Thus, some females may increase yolk A_4 levels with increasing communal clutch size and possibly improve the competitiveness of their offspring, when faced with a large number of nestmates. The eggs of female house sparrows *Passer domesticus* nesting at higher densities had higher concentrations of testosterone than those of females nesting in isolation, possibly due to more severe aggressive interactions among colony females (Schwabl, 1997). In tree swallows (*Tachycineta bicolor*), higher rates of aggressiveness among females before and during laying resulted in greater concentrations of testosterone in eggs (Whittingham and Schwabl, 2002), and the testosterone concentrations in eggs of European starlings depended on breeding density (Pilz and Smith, 2004).

Within communal nests, we also found that A_4 levels in first eggs were lower than in last eggs. Two explanations are likely. One is that there may be less competition at the onset of laying in a communal clutch. Guira cuckoo breeding groups are very dynamic and complex, and the number of adults may sometimes (though not commonly) vary during a nesting bout, from nest building to fledging of young (Macedo, 1992). Thus, at the beginning of laying, there may be fewer individuals in groups, and this number may increase as laying progresses, resulting in higher competition later in the nesting bout. An alternative explanation is that the lower overall investment in first eggs, including steroids, may minimize energetic losses due to egg ejection, which is prevalent at the onset of laying (Macedo, 1992; Macedo et al., 2004b).

In this study, we also analyzed the seasonal and ecological components of androgen deposition into guira cuckoo eggs. The lowest A_4 levels occurred in the relatively dry period of the reproductive season, when we found mostly small clutches, laid by fewer females (Macedo et al., 2004a). In contrast, the highest A_4 levels were found during the beginning of the rainy season, when we also found a greater number of large clutches. Correspondingly, we found that eggs had lower A_4 concentrations during first nesting bouts as compared to subsequent nesting attempts. First nestings, which are earlier in the breeding season, usually are composed of small clutches (Macedo et al., 2004a). Larger clutches occurred as the rains increased (period 2). This may be because the optimal time of egg hatching should coincide with peak abundances of insects (which in the study area occurred in period 3, later in the season) to meet the higher food demands by the chicks. Thus, the temporal component of androgen deposition into eggs may reflect the competitive scenario we described previously, wherein larger communal clutches represent higher levels of competition.

To our knowledge, androgen concentrations in eggs in a communal or cooperative breeder have never been examined. Such systems provide a natural framework to investigate the association between levels of competition among individuals and the possible effects of androgens in eggs. Based on our results, we propose that, for this species, with the reproductive characteristics inherent to a communal breeder with joint nesting, the variation among individual females in mean concentration of androgens in their individual clutches may function in adaptive ways. We suggest that the higher levels of A_4 in eggs

laid by individual females later in their clutches may function to increase the competitive abilities of chicks that have higher chances of hatching since these eggs are less likely to be ejected. The first eggs in the communal clutch are more likely to suffer ejection, and investment of androgens in these early eggs could be useless. This is further substantiated by the fact that the very first egg in communal clutches has lower A_4 when compared with the very last egg. We also propose that the positive association found between increasing communal clutch size and higher A_4 levels is directly related to the resulting elevated rates of competition among more numerous females and hatchlings. This is circuitously related to the A_4 seasonal variation we found. The larger communal clutch sizes found in the initial period of rains may reflect the higher food availability for chicks in the subsequent phase and constitute the optimum occasion for offspring production. Further studies are necessary to investigate other aspects, particularly those with respect to hormonal influence on offspring survival and development.

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