



Hormonal correlates of social status and courtship display in the cooperatively lekking lance-tailed manakin

Emily H. DuVal^{a,b,c,*}, Wolfgang Goymann^d

^a Department of Behavioural Ecology and Evolutionary Genetics, Max Planck Institute for Ornithology, Seewiesen, Germany

^b Museum of Vertebrate Zoology, University of California, Berkeley; 3101 Valley Life Sciences Bldg., Berkeley, CA 94720-3160, USA

^c Florida State University, Department of Biological Science, King Life Sciences Building, 319 Stadium Dr., Tallahassee, FL 32306-4295, USA

^d Abteilung für Verhaltensneurobiologie, Max-Planck-Institut für Ornithologie, Eberhard-Gwinner-Straße, D-82319 Seewiesen, Germany

ARTICLE INFO

Article history:

Received 22 July 2010

Revised 2 October 2010

Accepted 4 October 2010

Available online 13 October 2010

Keywords:

Chiroxiphia lanceolata

Manakin

Testosterone

Cooperation

Courtship

Lek

Androgen

ABSTRACT

Though cooperative behavior has long been a focus of evolutionary biology, the proximate hormonal mechanisms underlying cooperative interactions remain poorly understood. Lance-tailed manakins (*Chiroxiphia lanceolata*) are tropical passerines that form long-term male–male partnerships and cooperate in paired male courtship displays. To elucidate patterns of natural hormonal variation in relation to cooperation and reproductive behavior, we examined circulating androgen levels of male lance-tailed manakins in relation to social status, display behavior, and time of year. We found significantly higher circulating androgen levels in alpha-ranked (breeding) males compared to non-alpha adult males in the population. Beta males, which participated in courtship displays but did not copulate, had androgen levels indistinguishable from those of unpaired adult males that never displayed for females, suggesting that an elevated concentration of plasma testosterone in tropical lekking birds may be associated primarily with copulatory behavior or other status-specific traits, and not the performance of courtship display. Androgens decreased throughout the breeding season for males of all status categories. Interestingly, alphas that displayed for females in the observation session prior to sampling had lower androgen levels than alphas that did not display for females. This pattern may result from female discrimination against alpha males at display areas with high levels of social conflict among males, as social disruption is linked to elevated testosterone in many species. However, recent change of a display partner was not related to alpha androgen levels. We discuss alternative explanations and the possible implications of these results, and generate several testable predictions for future investigations.

© 2010 Elsevier Inc. All rights reserved.

Introduction

The evolution of cooperation has long been the subject of biological inquiry, and understanding the processes that maintain cooperative interactions in complex social groups remains a critical goal of evolutionary research (Clutton-Brock, 2002). The ultimate causes of cooperation in vertebrate social systems have attracted considerable interest, but physiological mechanisms underlying this behavior are less well-studied (Soares et al., 2010). With the goal of investigating a physiological correlate of cooperative behavior, we quantified variation in circulating androgen levels among breeders, helpers, and non-helpers in males of the cooperatively displaying lance-tailed manakin (*Chiroxiphia lanceolata*). This work not only investigates the proximate mechanisms of reproductive cooperation in a novel social context, but also provides much-needed information

about androgen profiles in tropical birds (see reviews in Goymann et al., 2004; Hau et al., 2008).

Steroid hormones represent important proximate mechanisms mediating variation in many male traits. Androgens such as testosterone influence individual variation in male traits, including levels of aggression, gamete production, and avian plumage, song and display (Adkins-Regan, 2005). Testosterone has been specifically implicated in maintaining high levels of aggression and courtship display observed in systems with strong sexual selection (Hirschenhauser and Oliveira, 2006), and experimental elevation of plasma testosterone increases display behavior in at least some lekking species (Day et al., 2006). Testosterone may also be important in cooperative interactions (Eisenegger et al., 2010; Soares et al., 2010). Androgens have been implicated as a key factor that varies between dominant breeders and subordinate helpers in several cooperatively breeding species. For example, reproductively inactive subordinate males have lower testosterone concentrations than reproductively active breeding males in a variety of species, including bell miners (*Manorina melanophrys*), Florida scrub jays (*Aphelocoma coerulescens*), Seychelle warblers (*Acrocephalus sechellensis*), and white-browed sparrow weavers

* Corresponding author. Current address: Florida State University, Department of Biological Science, King Life Sciences Building, 319 Stadium Dr., Tallahassee, FL 32306-4295, USA. Fax: +1 850 645 8447.

E-mail address: ehduval@bio.fsu.edu (E.H. DuVal).

(*Plocepasser mahali*) (Brouwer et al., 2009; Poiani and Fletcher, 1994; Schoech et al., 1991, 1996; Wingfield et al., 1991). In cooperative breeders in which subordinates regularly reproduce, differences in testosterone concentrations between dominants and subordinates are less pronounced, as found in Mexican jays (*Aphelocoma ultramarina*), Harris' hawks (*Parabuteo unicinctus*) and Australian magpies (*Cracticus tibicen*), (Mays et al., 1991; Schmidt et al., 1991; Vleck and Brown, 1999). In keeping with this trend, secondary helpers in pied kingfisher (*Ceryle rudis*) groups, which are unrelated to the breeding pair and represent a reproductive threat to breeding males, have testosterone levels similar to those of breeders, whereas primary helpers, which are offspring of the breeding pair, express low levels of testosterone (Reyer et al., 1986). However, helpers of some cooperative species have levels of testosterone similar to those of breeders, even though they appear to be reproductively inactive, as in azure-winged magpies (*Cyanopica cyana*) and red-cockaded woodpeckers (*Picoides borealis*) (de la Cruz et al., 2003; Khan et al., 2001). In contrast, subordinate male superb fairy wrens (*Malurus cyaneus*) are reproductively active, but have lower levels of testosterone than dominants (Peters et al., 2001). A recent review concluded that while there are some trends in testosterone patterns of cooperative breeders, definite conclusions may be hindered by the complexity and subtle differences in the social systems summarized under the header "cooperative breeding" (Schoech et al., 2004).

Cooperative breeding is usually characterized by alloparental care of offspring (care provided by non-parental adults) (Stacey and Koenig, 1990), and therefore interpretation of androgen expression in cooperative breeders may be complicated by parental care interactions. For example, dominant breeders may not express testosterone at maximal levels throughout the breeding season, because typically males express low levels of testosterone during parental care (Wingfield et al., 1990), although this pattern is not universal (reviewed in Goymann, 2009; Lynn, 2008). Seasonal patterns of testosterone expression in non-cooperative species have similarly been attributed to conflicting demands of courtship and parental care (Chastel et al., 2005). In contrast, individuals that provide no parental care for young, such as lekking males, should demonstrate androgen profiles independent of these restrictions proposed for traditional cooperative breeders. In at least one lekking species, seasonal trends in testosterone expression are clear despite the lack of parental care (Day et al., 2006). Individuals emancipated from parental duties but that nevertheless cooperate in courtship therefore provide a useful opportunity to investigate the relationship of cooperative behavior and androgen levels. Though other studies have investigated hormonal correlates of cooperative breeding and of courtship display, this study, to our knowledge, represents the first quantification of hormone levels in a cooperatively displaying species.

Androgen levels of tropical birds have come under recent scrutiny as researchers reassess the generalization that tropical birds experience low androgen levels, even at the peak of breeding (Hau, 2001; Levin and Wingfield, 1992; Wikelski et al., 2003). Instead, recent work indicates that testosterone levels of tropical birds may peak at levels comparable to those of their temperate counterparts; and that the length of the breeding season has a strong influence on seasonal variation in circulating testosterone (Goymann et al., 2004; Hau et al., 2008). Relatively few lek-polygynous species have been studied in the tropics, with the notable exception of the golden-collared manakin (*Manacus vitellinus*). Golden-collared manakins experience elevated testosterone levels at the start of the courtship period and low testosterone in the non-breeding season, but testosterone levels fluctuate during the breeding season apparently in response to interactions with females and other males (Day et al., 2007; Fusani et al., 2007; Schlinger et al., 2008; Wikelski et al., 2003). Exogenous testosterone activates courtship display in golden-collared manakins outside the breeding season and even in females (Day et al., 2006) and treatment with androgen blockers decreases display behavior at least

for a short period of time (Fusani et al., 2007). In comparison, testosterone levels in temperate lekking species generally remain high throughout the relatively short courtship period (Alatalo et al., 1996; Mougeot et al., 2005). Male golden-collared manakins display competitively and in close proximity to one another, but courtship behavior in the manakin family ranges from solitary display to complex cooperative dances (Prum, 1994; Sick, 1967). It is unclear how trends reported in a classically lekking species may be modulated by the cooperative alliances that characterize some manakin species.

Lance-tailed manakins display for females in an exploded lek mating system, using cooperative male displays (DuVal, 2007b). Male partners form long-term alliances in which they sing duet songs and, when females are present, perform dances that involve coordinated leaps over one another and slow flight displays around the display perch on which the female sits. Males may maintain specific partnerships for up to five consecutive years (DuVal, 2007c). Adult males may be alphas (potential breeders), betas (non-breeding helpers that cooperate in courtship displays), or non-helpers (affiliate males that do not participate in courtship). Lance-tailed manakins do not maintain traditional territories, instead allowing conspecific males to enter their display area under most circumstances. Though all adult males may interact in dance and song displays when no females are present, only alphas and betas display for females, and only alpha males perform solo courtship displays or copulate. This pattern of status-specific display behavior and copulatory success has been confirmed previously through extensive observation of behavior at lek display sites, and through genetic paternity tests identifying successful sires in the study population (DuVal, 2007a; DuVal and Kempnaers, 2008).

With the goal of understanding the physiological correlates of cooperation and mate choice in the lance-tailed manakin, we first investigated how androgen levels vary among different status classes of males, exploring the potential association of courtship display or mating behavior with androgen levels. Second, we examined seasonal patterns of androgen expression, testing the hypothesis that the extended tropical breeding season results in prolonged elevation of androgens when there are no tradeoffs with parental care. Finally, we investigated how androgen levels relate to courtship display and social disruption. Although the data presented here reflect males' natural hormonal profiles, this study provides the necessary observational foundation for future manipulative work.

Materials and methods

Field methods

The study was conducted on a wild population of lance-tailed manakins found on Isla Boca Brava, Chiriquí Province, Republic of Panamá (8°12'N, 82°12'W), with the permission of local authorities and under animal care and use guidelines of the University of California, Berkeley. This site is a 46 ha area of secondary growth, dry tropical forest (<100 m above sea level). Blood samples for hormone analysis were collected from 2001 to 2003, during the breeding season (March–June) in each year; females visit male display areas and copulate throughout this time period (DuVal, 2007b).

Birds were captured with mistnets, marked with a unique combination of colored plastic leg bands, and bled for hormonal analyses. Blood samples of approximately 80 µl were collected in heparinized capillary tubes from the brachial wing vein within 10 min of removal from the net. It is widely acknowledged that handling stress may increase circulating androgen levels, and recent evidence suggests that these changes may be more rapidly induced than previously appreciated (Deviche et al., 2010; Van Hout et al., 2010). Though time between extraction from the net and blood sampling was unrelated to androgen levels in the subset of males for which this information was recorded (mean handling time for this sample was

7.7 ± 5.8 min; Pairwise correlation: $r = -0.27$, $p = 0.22$, $N = 23$ males), time of initial entanglement in the net was not directly observed and therefore we lack information on the precise degree of capture stress birds experienced before sampling. However, we have no reason to believe that there was any systematic variation in handling times that could have produced a bias in the results presented here. Tubes were sealed at both ends with Critoseal (Fisher Scientific, USA) and stored on ice until centrifugation. Samples were centrifuged at 2000 $\times g$ and the plasma fraction separated by breaking the capillary tube at the red cell-plasma interface. Plasma was then blown into a clean 1.5 mL screw-top vial and stored in liquid nitrogen.

This species exhibits two distinctive subadult-plumages, which allow unambiguous classification of age for young males. Males in their second year after hatching have a red cap both are otherwise green, while third-year males retain the red cap but additionally have a black face, mottled black-and-green body and flight feathers, and scattered blue on an otherwise green back (DuVal, 2005). Males attain definitive adult plumage, which consists of a red crest, a blue back, and black body and flight feathers, in the breeding season of their fourth year after hatching. Only males in definitive adult plumage perform courtship displays for females.

Behavioral observations were performed at male display areas for 2-hour periods three days a week. Observers recorded all occurrences of duet songs and dance displays (noting presence or absence of females and timing all songs or displays). Male status was determined by observation of color-banded birds at display areas, and a suite of status-correlated behaviors were used to statistically identify alpha, beta, and nonpair (non-helper) males independently of reproductive success (DuVal, 2007c). This method of status assignment is described exhaustively elsewhere (DuVal, 2007c), but in short, the alpha male at each display area was the most commonly present male, participated in the vast majority of duet songs that occurred in the area, and was the only male to perform solo displays for females. The beta male was the alpha's most common duetting partner, and participated in paired displays for females but not solo courtship displays. Other adult-plumage males that were neither alpha nor beta (here referred to as "non-helper" males) occasionally participated in duet songs, or participated in dances when no females were present. Display areas were generally consistent in location across years, and the same males frequently held the same social status at a given display area for several years. Partner switches were identified by the disappearance and replacement of a male previously identified as alpha or beta at a given display area.

Courtship display for females is usually initiated as a "pip flight" display: when a female approaches the display perch, the alpha male circles the perch area repeating a distinctive "pip" call, sometimes in coordination with the beta partner (DuVal, 2007b). If the female then follows the male to the display perch, the courtship proceeds to a full male "dance", including butterfly-like slow flights, a backwards leapfrog display by the alpha and beta partners, and up to seven other display elements, described in detail elsewhere (DuVal, 2007b). For the purposes of this paper, both displays that terminated as pip flights and those that proceeded to more complete displays are all classified as courtship displays for females.

Radioimmunoassay of testosterone

Androgen concentration was determined by direct radioimmunoassay (RIA, following Goymann et al., 2006). Plasma samples were extracted with dichloromethane (DCM) after overnight equilibration (4 °C) of the plasma with 1500 dpm of tritiated testosterone (Perkin Elmer, Wellesley, MA, USA). The organic phase was then separated from the aqueous phase by plunging the extraction tubes into a methanol-dry ice bath and decanting the dichloromethane phase into a new vial. This extraction step was repeated twice to increase extraction efficiency. Then, the DCM phase was dried under a stream

of nitrogen at 40 °C, dried samples were resuspended in phosphate buffered saline with 1% gelatine (PBSG) and left overnight at 4 °C to equilibrate. An aliquot (80 μ l) of the redissolved samples was transferred to scintillation vials, mixed with 4 ml scintillation fluid (Packard Ultima Gold) and counted to an accuracy of 2–3% in a Beckman LS 6000 β -counter to estimate individual extraction recoveries. The remainder was stored at –40 °C until RIA was conducted. Mean $\pm$ sd extraction efficiency for plasma testosterone was $89.0 \pm 4.2\%$. Samples were analyzed in two RIAs. For each RIA a standard curve was set up in duplicates by serial dilution of stock standard testosterone ranging from 0.39 to 200 pg. Testosterone antiserum (T3-125, Esoterix Endocrinology, Calabasas, CA, USA) was added to the standard curve, the controls and to duplicates of each sample (100 μ l). After 30 min testosterone label (13,500 dpm) was added and the assay incubated for 20 h at 4 °C. Bound and free fractions were then separated at 4 °C by adding 0.5 ml dextran-coated charcoal in PBSG assay buffer. After 14 min incubation with charcoal samples were spun (3600 g, 10 min, 4 °C) and supernatants decanted into scintillation vials at 4 °C. After adding 4 ml scintillation liquid (Packard Ultima Gold), vials were counted. Standard curves and sample concentrations were calculated with Immunofit 3.0 (Beckman Inc. Fullerton, CA), using a four parameter logistic curve fit. The lower detection limit of each standard curve was determined as the first value outside the 95% confidence intervals for the zero standard ($B_{\max}$) and was 0.7 pg/tube. The intra-assay coefficients of variation were 4.8 and 2.3% and the extraction coefficient of variation was 1.5%. The inter-assay variation was 12.4%. Because the testosterone antibody used shows significant cross-reactions with 5 α -dihydrotestosterone (44%) our measurement may include a fraction of 5 α -DHT. We therefore refer to quantified androgens rather than testosterone throughout the paper.

Statistical analysis

Analyses were performed using general linear models in the programs JMP and R 2.10.0, using a significance level of $\alpha = 0.05$. Androgen concentration was $\log_{10}$ -transformed for normality. For individuals with more than one plasma sample collected ($N = 14$ individuals), one sample was randomly selected for analysis as the number of repeatedly sampled individuals was insufficient for a mixed model approach; the others were excluded from all summary statistics as well as results. For individuals sampled in multiple status categories, the sample with the highest associated social status for that individual was retained. All years are combined in these analyses as no significant differences in testosterone concentrations of adult males were detected among years (ANOVA: $F_{(2,61)} = 0.84$ $p = 0.44$).

Results

Plasma androgen levels were quantified for 13 alpha males, 13 beta (helper) males, and 38 adult-plumage males that were neither alphas nor betas. We additionally sampled 23 males in subadult plumage (15 third-year and 8 second-year males). Plasma androgen levels for all males ranged from 52 pg/ml to 4.2 ng/ml.

Androgen levels in subadult males

Subadult-plumage males (males in their second and third year after hatching) had significantly lower androgen levels than adult-plumage males (t-test: $t_{(85)} = 2.47$, $p = 0.02$; Fig. 1). There was no difference in androgen levels between males in the second- and third-year subadult-plumage classes (t-test, $t_{(21)} = 0.30$, $p = 0.77$). As subadult males do not sire chicks and are not involved in courtship displays for females (DuVal and Kempnaers, 2008), subadults were excluded from all subsequent analyses.

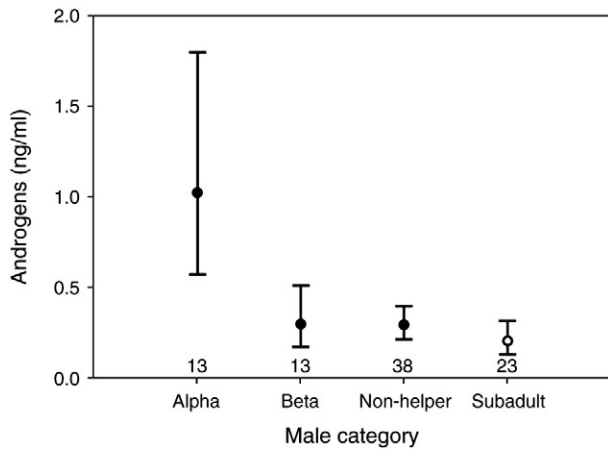


Fig. 1. Alpha males had significantly higher plasma androgen levels than other males in the population. Beta males, which cooperate in courtship displays for females with alpha partners, had androgen levels indistinguishable from those of adult males not in a display partnership (males of “non-helper” social status). Males in subadult-plumage (second and third year after hatching) had lower androgen levels than adult-plumage males. Back-transformed means with 95% confidence intervals are shown; sample sizes are indicated below each bar.

Androgen levels and adult social status

Androgen levels in adult-plumage males were significantly related to individuals' social status (ANOVA: $F_{2,61} = 9.34$, $p < 0.001$; Fig. 1). Alpha males had higher plasma androgen levels than either beta or non-helper adult males (Tukey's HSD, $p < 0.05$). All other comparisons were non-significant. Androgen differences may be the result of dominance interactions associated with taking over breeding status, rather than the result of the status itself. We therefore hypothesized that males should have higher androgen levels in their first breeding season after assuming alpha status compared to those that have held alpha position for longer periods of time. However, males known to be in their first year of alpha status did not differ in plasma androgens when compared with alphas that had held their current status for two or more breeding seasons ($t_{11} = 0.96$, $p = 0.36$, $N = 5$ first-year alphas, 8 alphas with longer tenure).

Seasonal effects in adult males

A seasonal decrease in circulating androgen levels was observed among adult males (linear regression: $F_{(1,62)} = 12.66$, $p < 0.001$; Fig. 2). Both this seasonal effect and the effect of male status reported

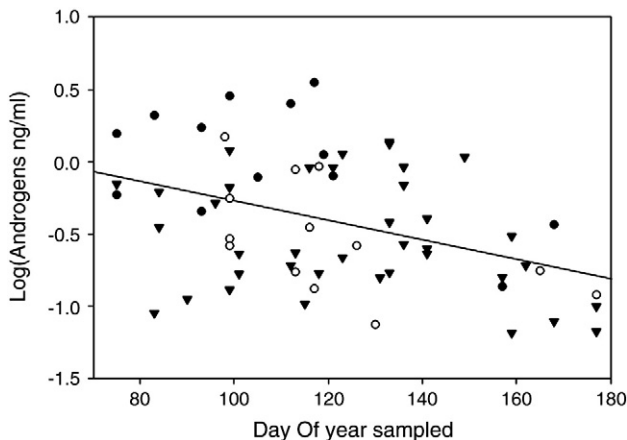


Fig. 2. Plasma androgen levels of definitive-plumage males regressed against day of year captured. $N = 13$ alpha males (filled circles), 13 beta males (open circles), and 38 males that were neither alphas nor betas (triangles).

previously remained significant when analyzed together in a generalized linear model (GLM: day of year captured, $F_{1,60} = 8.75$, $p = 0.004$; status $F_{2,60} = 7.29$, $p = 0.002$). We detected no significant interaction between the effects of status and sampling date on androgen levels, though low sample size in each status category limits our ability to detect such a relationship. This seasonal effect was strongly influenced by lower androgen levels of males sampled after day 160 (early June); excluding these males resulted in no significant relationship of sampling date with androgen levels (linear regression: $F_{1,50} = 0.32$, $p = 0.5$; status $F_{2,60} = 7.29$, $p = 0.002$).

Post-hoc examination of parameter estimate confidence intervals suggests that seasonal declines in androgen levels are largely driven by declines in androgens of alpha and beta males, and the lack of detectable interaction is likely the result of small sample sizes in individual status groups. When analyzed separately, both alpha and beta males showed a non-significant decrease in androgen levels with sampling date, with a similar regression coefficients (Linear regressions: alphas, $F_{(1,11)} = 3.99$, $p = 0.07$, regression coefficient = -0.007 ± 0.004 ; betas, $F_{(1,11)} = 4.3$, $p = 0.06$, regression coefficient = 0.008 ± 0.004).

Androgen levels and courtship display

Among alpha males, androgen levels were related to the occurrence of courtship displays for females (pip flight or dance displays). Alpha males that displayed for a female in the observation session prior to plasma sampling had lower androgen levels than those males that did not display for females during the observation session prior to sampling ($t_{11} = 4.67$, $p < 0.001$; Fig. 3; observations sessions were 3.5 h to 4 days before capture; average = 1.9 ± 1.0 days). Male lance-tailed manakins also performed dance displays when no females were present, but the occurrence of dance displays with no females present in the observation session before sampling was unrelated to alpha androgens ($t_{11} = -0.618$, $p = 0.54$, $N = 13$). Similarly, there was no relationship between alpha androgen levels and the rate of male–male duet singing in the session prior to sampling ($F_{1,11} = 0.05$, $p = 0.83$). Betas at display areas where courtship displays for females were observed in the observation session prior to plasma sampling had androgen levels not significantly different from those of betas at areas where no courtship display occurred ($t_{11} = 0.26$, $p = 0.8$).

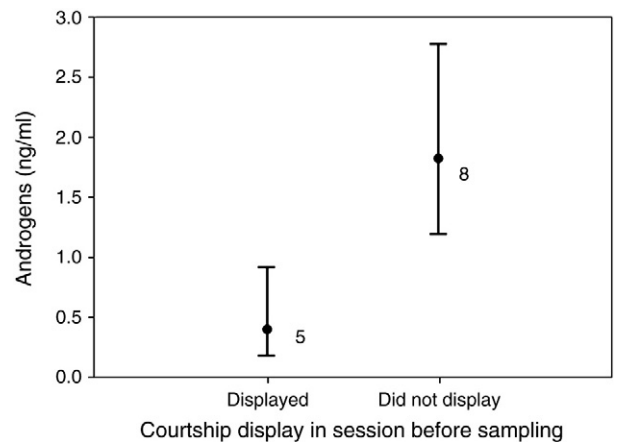


Fig. 3. Alpha male display behavior was related to individual variation in androgen levels. Alpha males observed performing courtship displays for a female on the dance perch in the observation session prior to plasma sampling had significantly lower androgen levels than alpha males that were not observed displaying for females. Observation sessions were 4 h to 4 days prior to capture and plasma sampling. $N = 13$ alpha males; sample sizes of subgroups shown. Back-transformed means with 95% confidence intervals are shown, with sample sizes indicated.

Androgen levels and social disruption

We compared androgen levels of males in display partnerships in which one partner had been replaced since the prior breeding season to that of males that had maintained their alliance from the previous year. Males that changed partners had androgen levels that were not significantly different from males that continued to display with previous partners (Alphas and betas: $t_{22} = 0.16$, $p = 0.87$; alphas only: $t_9 = -0.13$, $p = 0.90$; betas only: $t_{11} = 0.44$, $p = 0.67$; 6 of 11 alphas with known histories had a new partner in the year sampled, and the same was true for 7 of 13 betas).

Discussion

In this study we found that alpha male lance-tailed manakins exhibit higher levels of circulating androgens during the breeding season than do beta males and non-displaying adult males. Surprisingly, beta male lance-tailed manakins exhibit low levels of circulating androgens, despite their participation in elaborate cooperative courtship displays and close interaction with females during those displays. Testosterone stimulates the performance of courtship display in many species, including in other manakins (Day et al., 2006). It is therefore curious that beta male lance-tailed manakins, which do regularly participate in courtship displays, have androgen levels indistinguishable from other non-alpha males in the population. This finding suggests that high androgen levels are not required for the expression of cooperative courtship behavior in this species.

In contrast to their subordinate helpers, androgen levels of alpha males were relatively high. This is consistent with the generalization that breeding males have higher testosterone levels than non-breeding helper males in most cooperatively breeding species (Schoech et al., 2004). Although both alpha and beta male lance-tailed manakins display for females, the behavior of alpha and beta manakins varies in many ways in addition to the performance of courtship displays. Alphas show increased vigilance and perform early courtship attraction displays for females near their display perches (“pip flight” displays), and alphas participate in more cooperative duet songs and sing with more male partners than do betas (DuVal, 2007c). In successful displays including dual male courtship dances, a solo display always precedes copulation and only alpha males have been observed to perform these late-courtship solo displays (DuVal, 2007c). Genetic tests have previously confirmed that alphas sire almost all chicks (DuVal and Kempenaers, 2008). Elevated androgen levels in alpha males relative to betas indicate that these alpha-specific behaviors or an increase in overall alpha male display time relative to beta males have a greater influence on individual androgens (or vice versa) than does the performance of cooperative courtship displays. Interestingly, androgen variation among adult males of different social classes may in part explain variation reported among breeding season adults in other manakin species (e.g. Day et al., 2007), for which social divisions are less immediately apparent but may nevertheless be present.

Comparison of the physiology of alpha and beta males in cooperative display partnerships specifically provides insights as to how hormones implicated in cooperation relate to display behavior independent of selective pressures relating to offspring care. It has previously been noted that species vary in their responsiveness to testosterone during periods of parental care (Hirschenhauser and Oliveira, 2006; Lynn, 2008). This study, in contrast, highlights the occurrence of seasonal decreases in androgen expression despite a lack of parental care.

Seasonality of androgen expression

Although androgen levels in alpha males were elevated, there was a general trend among all males for a decrease in androgens as the

breeding season progressed. This trend is particularly influenced by lower androgen levels detected after day 160 (early June), raising the possibility that androgens drop steeply at the end of the breeding season rather than declining throughout the breeding period. Display rates were generally lower in June than in late March, though breeding activity was ongoing at this time of year. Systematic behavioral observations showed that alpha and beta males were still present and active on their display areas in June. Displays for females, including displays that ended with copulations, were observed until fieldwork ended in late June, indicating that the breeding season had not ended before day 160 (DuVal, 2007b). Our ability to make strong inferences about seasonal changes in the hormone profile of individual birds is limited by the cross-sectional nature of our sample. However, a longitudinal study of individual golden-collared manakin males sampled at the start of the breeding period and again one month later also showed a decrease in testosterone, though there was no change in courtship frequency in this time period (Fusani et al., 2007). A recent study of seasonal modulation of testosterone in Costa Rican rufous-collared sparrows (*Zonotrichia capensis costaricensis*) also showed a seasonal decline of testosterone independent of breeding condition (Addis et al., 2010), and tropical spotted antbirds (*Hylophylax n. naevioides*) maintain courtship and reproductive behaviors even when testosterone levels are baseline (Wikelski et al., 1999), perhaps in concert with seasonal changes in hormone receptor sensitivity (Canoine et al., 2007). The cross-sectional sample reported in this study of lance-tailed manakins suggests a seasonal decline in testosterone as reported in other tropical species; a decrease in androgens throughout the breeding season despite the maintenance of high levels of courtship display may be a general trend in lekking manakins.

Considered with the previous evidence from beta androgen levels, which suggests that elevated testosterone is not required for the performance of courtship display, a seasonal decline in androgen levels would further suggest that naturally elevated levels of androgens may function primarily in the establishment of display territories early in the breeding season, or other early season social interactions rather than directly influencing courtship behavior during the breeding season. Alternatively, certain levels of androgens may be required to activate display behavior at the beginning of the breeding season, but sustained high levels may not be required to actually display (see also Fusani et al., 2007).

Testosterone in tropical species

Androgen values of alpha males were comparable to average testosterone concentrations reported for many other tropical bird species (Goymann et al., 2004). Male lance-tailed manakins apparently remain on their display territories year-round, though breeding activity peaks during a breeding season that lasts roughly four months (DuVal, 2007b). The observed androgen levels were similar to values, given a long breeding season, predicted in a meta-analysis of testosterone in tropical birds (Goymann et al., 2004).

A number of hypotheses have been proposed to explain the general trend of decreased testosterone in tropical relative to temperate species. Of the multiple social factors proposed to contribute to low testosterone concentrations in tropical birds, long breeding season, long-term social stability, year-round territoriality, “slow” life history, and cooperative group interactions may all moderate androgen levels in lance-tailed manakins. Several other hypotheses can be rejected a priori as inapplicable to this study species, as lance-tailed manakins do not have a monogamous mating system, long-term male–female pair bonds, or any male parental care, all of which have been proposed as factors moderating androgen expression (Hau et al., 2008). Social interactions in cooperatively breeding birds may have an important influence on circulating androgen levels and may underlie the observation that overall testosterone in breeding males of tropical cooperatively breeding

species is lower than that of monogamous or polygamous species (Hau et al., 2008). Similar to cooperative breeding species, males of cooperatively displaying species form close social alliances with other males, experience long-term relationships with other males, and finely adjust their interactions with these males to maintain these partnerships.

Androgen and social status in cooperatively displaying males

What explains the differences in androgen concentrations observed between alpha and beta males? Two of the major sources of variation in individual androgen levels that may explain differences between alpha and beta males are the effects of age or the effects of social environment (Kempnaers et al., 2008). Intrinsic individual effects are less likely than extrinsic factors to influence the observed correlation of social status and testosterone levels, as individual beta lance-tailed manakins often progress to alpha status over time (DuVal, 2007a). Age differences between helpers and breeders are also suggested as one explanation for different levels of testosterone expression (Schoech et al., 2004). In keeping with this, we found that young (subadult-plumage) male lance-tailed manakins did show decreased androgen levels compared with older (adult-plumage) males; similar results have been found in other manakin species (Day et al., 2007). Alphas are significantly older than their beta partners, though some alphas are younger than some betas in the population, and some unpaired males are older than some alphas (DuVal, 2007c). Due to limited knowledge of the age of the adult males sampled in this study, we are unable to test directly for interactions of age and androgen levels among alpha and beta males. Age-linked physiological changes will be an important variable to address in future work.

Testosterone is proposed to be an important mediator of life history tradeoffs, for example between investments in survival versus reproduction. Testosterone carries a suite of potential costs associated with high levels of expression. For example, in Red Grouse (*Lagopus lagopus scoticus*), elevated testosterone levels were linked to increased parasite loads and oxidative stress (Mougeot et al., 2009). If elevated testosterone functions primarily in physiological pathways relevant only to alpha males (e.g. sperm production in a system where access to females is socially restricted), reduced expression of androgens may allow non-alpha males to avoid incurring these costs unnecessarily. Interestingly, average androgen concentrations of breeding alpha lance-tailed manakins were similar to those of polygamous tropical birds, while beta males exhibited testosterone levels similar to those of males in cooperatively breeding species (Hau et al., 2008).

Androgens and alpha male courtship display

Although there is substantial evidence that the presentation of reproductively active females increases androgen secretion in a variety of birds (reviewed in Goymann, 2009), we found that alpha males that displayed for females before plasma sampling had reduced androgen levels compared to alpha males that did not. The current observational study lacks the ability to separate cause and effect in this relationship, but suggests several interesting avenues for further study. We emphasize that the observation of relatively low androgen levels among alpha males that successfully attract females may be an artifact of the small sample size of this study, and additional sampling is necessary to test the generality of this relationship.

However, if the relationship is upheld, several hypotheses could explain the observed relationship. First, there may be asynchronous peaks of male–male interactions and displays for females. If androgen increases are driven primarily by competitive interactions with other males rather than courtship behavior for females, and if male–male interactions are most common early in the breeding season, then male androgen levels may decline as female visitation increases after the start of the breeding season. This predicts that the observed effect will

not be evident in alpha males sampled late in the breeding season. In the current study, alpha males that displayed for females were sampled slightly later in the breeding season (mean day of year sampled: 123 ± 40 days) than alphas that did not display for females (mean day of year sampled: 100 ± 16 days), though this difference was not significant (Wilcoxon Rank Sums test: $Z = 0.95$, $p = 0.34$; range = day 75–119 for non-displaying alphas, day 75–168 for displaying alphas). Furthermore, roughly half of the testosterone samples were collected in the year 2001, in which there was no significant decline in courtship frequency with day of the breeding season, unlike other years of the study (DuVal, 2007b). In two cases when males in each category were sampled on the same day of the year, the alpha that recently displayed for a female had lower testosterone than the non-displaying male, suggesting that the low testosterone values of displaying alphas cannot be explained by seasonal effects alone.

A second possibility is that there may be an antagonistic response of androgens to different types of social stimuli. Male–male interactions can lead to decreases in androgen levels in some species (Goymann et al., 2007; Landys et al., 2007), and in theory the same could be true of male–female interactions in the lance-tailed manakin. In this scenario, lower levels of androgens should be observed in males following courtship display for females, but not preceding such display.

Finally, this relationship could reflect female discrimination against alpha males in unstable social situations. If androgen levels are elevated in situations of social conflict, and females prefer males in stable social alliances as has been reported in the close-related long-tailed manakin (*Chiroxiphia linearis*) (McDonald, 1993), then the reported trend may reflect female avoidance of unstable male pairs rather than an induced decrease in males' androgen levels following exposure to females.

We detected no significant effect of relatively recent takeover of alpha status on male androgen levels. However, changes in androgen expression that are predicted due to male–male interactions should be restricted to times during which males are actively establishing their new display areas (Wingfield et al., 1990), whereas our measures of androgen levels included males that were already established in the alpha role (albeit for the first breeding season), not those actively undergoing a status shift. It is therefore unsurprising that we detected no difference in androgen levels among these classes of males.

General conclusions

In summary, we found marked differences in androgen levels among alpha and non-alpha male lance-tailed manakins, and detected a general decline in androgen levels throughout the breeding season. An inverse relationship of alpha androgen level and the occurrence of courtship displays for females warrant further investigation, particularly in light of predictions from studies of a congeneric manakin which suggest that females discriminate against males in unstable social alliances; social instability is commonly correlated with increased frequency of aggressive interactions, which may influence androgen levels of the males involved. To our knowledge, these results represent the first quantification of androgen levels in a cooperatively displaying species, and suggest that high levels of androgen expression are not required for male performance of complex courtship display. These data underscore findings from more traditional cooperative breeders showing that breeders generally have higher testosterone than non-breeding helpers. Finally, seasonal androgen levels decrease despite the lack of male parental care in this species, suggesting that factors such as frequent male–male interactions early in the breeding season rather than antagonistic effects of testosterone and parental care after hatching may explain a greater amount of seasonal androgen trends than previously appreciated.

Acknowledgments

We thank Ingrid Schwabl for performing radio-immunoassays, and Rebecca Carter, Katie Janaes, Ryan Lorenz, Julie Lorion, and Erik Reeder for field assistance related to the collection of this data. Bart Kempnaers provided support and stimulating discussion during the development of this work, and Julian Kapoor provided useful comments on a previous draft of this paper. This project was funded in part by a Society for Integrative and Comparative Biology Grant-in-Aid of Research to EHD.

References

- Addis, E.A., Busch, D.S., Clark, A.D., Wingfield, J.C., 2010. Seasonal and social modulation of testosterone in Costa Rican rufous-collared sparrows (*Zonotrichia capensis costaricensis*). *Gen. Comp. Endocrinol.* 166, 581–589.
- Adkins-Regan, E., 2005. Hormones and animal social behavior. Princeton University Press, Princeton, NJ.
- Alatalo, R.V., Hoglund, J., Lundberg, A., Rintamäki, P.T., Silverin, B., 1996. Testosterone and male mating success on the black grouse leks. *Proc. R. Soc. Lond. B* 263, 1697–1702.
- Brouwer, L., Groothuis, T.G.G., Vedder, O., Eikenaar, C., Richardson, D.S., Komdeur, J., 2009. Do primary males physiologically suppress subordinate males? An experiment in a cooperatively breeding passerine. *Ethology* 115, 576–587.
- Canoine, V., Fusani, L., Schlinger, B., Hau, M., 2007. Low sex steroids, high steroid receptors: Increasing the sensitivity of the nonreproductive brain. *Dev. Neurobiol.* 67, 57–67.
- Chastel, O., Barbraud, C., Weimerskirch, H., Lormee, H., Lacroix, A., Tostain, O., 2005. High levels of LH and testosterone in a tropical seabird with an elaborate courtship display. *Gen. Comp. Endocrinol.* 140, 33–40.
- Clutton-Brock, T., 2002. Breeding together: Kin selection and mutualism in cooperative vertebrates. *Science* 296, 69–72.
- Day, L.B., McBroom, J.T., Schlinger, B.A., 2006. Testosterone increases display behaviors but does not stimulate growth of adult plumage in male golden-collared manakins (*manacus vitellinus*). *Horm. Behav.* 49, 223–232.
- Day, L.B., Fusani, L., Hernandez, E., Billo, T.J., Sheldon, K.S., Wise, P.M., Schlinger, B.A., 2007. Testosterone and its effects on courtship in golden-collared manakins (*Manacus vitellinus*): Seasonal, sex, and age differences. *Horm. Behav.* 51, 69–76.
- de la Cruz, C., Solis, E., Valencia, J., Chastel, O., Sorci, G., 2003. Testosterone and helping behavior in the azure-winged magpie (*Cyanopica cyanus*): Natural covariation and an experimental test. *Behav. Ecol. Sociobiol.* 55, 103–111.
- Deviche, P.J., Hurley, L.L., Fokidis, H.B., Lerbour, B., Silverin, B., Sabo, J., Sharp, P.J., 2010. Acute stress rapidly decreases plasma testosterone in a free-ranging male songbird: Potential site of action and mechanism. *Gen. Comp. Endocrinol.* 169, 82–90.
- DuVal, E.H., 2005. Age-based plumage changes in the lance-tailed manakin: a two-year delay in plumage maturation. *Condor* 107, 917–922.
- DuVal, E.H., 2007a. Adaptive advantages of cooperative courtship for subordinate male lance-tailed manakins. *Am. Nat.* 169, 423–432.
- DuVal, E.H., 2007b. Cooperative display and lekking behavior of the lance-tailed manakin (*Chiroxiphia lanceolata*). *Auk* 124, 1168–1185.
- DuVal, E.H., 2007c. Social organization and variation in cooperative alliances among male lance-tailed manakins. *Anim. Behav.* 73, 391–401.
- DuVal, E.H., Kempnaers, B., 2008. Sexual selection in a lekking bird: The relative opportunity for selection by female choice and male competition. *Proc. R. Soc. Lond. B* 275, 1995–2003.
- Eisenegger, C., Naef, M., Snozzi, R., Heinrichs, M., Fehr, E., 2010. Prejudice and truth about the effect of testosterone on human bargaining behaviour. *Nature* 463, 356–359.
- Fusani, L., Day, L.B., Canoine, V., Reinemann, D., Hernandez, E., Schlinger, B.A., 2007. Androgen and the elaborate courtship behavior of a tropical lekking bird. *Horm. Behav.* 51, 62–68.
- Goymann, W., 2009. Social modulation of androgens in male birds. *Gen. Comp. Endocrinol.* 163, 149–157.
- Goymann, W., Moore, I.T., Scheuerlein, A., Hirschenhauser, K., Grafen, A., Wingfield, J.C., 2004. Testosterone in tropical birds: effects of environmental and social factors. *Am. Nat.* 164, 327–334.
- Goymann, W., Trappschuh, M., Jensen, W., Schwabl, I., 2006. Low ambient temperature increases food intake and dropping production, leading to incorrect estimates of hormone metabolite concentrations in European stonechats. *Horm. Behav.* 49, 644–653.
- Goymann, W., Landys, M.M., Wingfield, J.C., 2007. Distinguishing seasonal androgen responses from male–male androgen responsiveness – revisiting the challenge hypothesis. *Horm. Behav.* 51, 463–476.
- Hau, M., 2001. Timing of breeding in variable environments: tropical birds as model systems. *Horm. Behav.* 40, 281–290.
- Hau, M., Gill, S.A., Goymann, W., 2008. Tropical field endocrinology: Ecology and evolution of testosterone concentrations in male birds. *Gen. Comp. Endocrinol.* 157, 241–248.
- Hirschenhauser, K., Oliveira, R.F., 2006. Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. *Anim. Behav.* 71, 265–277.
- Kempnaers, B., Peters, A., Foerster, K., 2008. Sources of individual variation in plasma testosterone levels. *Philos. Trans. R. Soc. B Biol. Sci.* 363, 1711–1723.
- Khan, M.Z., McNabb, F.M.A., Walters, J.R., Sharp, P.J., 2001. Patterns of testosterone and prolactin concentrations and reproductive behavior of helpers and breeders in the cooperatively breeding red-cockaded woodpecker (*Picoides borealis*). *Horm. Behav.* 40, 1–13.
- Landys, M., Goymann, W., Raess, M., Slagsvold, T., 2007. Hormonal responses to male–male social challenge in the blue tit cyanistes caeruleus: single-broodedness as an explanatory variable. *Physiol. Biochem. Zool.* 80, 228–240.
- Levin, R.N., Wingfield, J.C., 1992. The hormonal control of territorial aggression in tropical birds. *Ornis Scand.* 23, 284–291.
- Lynn, S.E., 2008. Behavioral insensitivity to testosterone: Why and how does testosterone alter paternal and aggressive behavior in some avian species but not others? *Gen. Comp. Endocrinol.* 157, 233–240.
- Mays, N.A., Vleck, C.M., Dawson, J., 1991. Plasma luteinizing hormone, steroid hormones, behavioural role, and nest stage in cooperatively breeding Harris' hawks (*Parabuteo unicinctus*). *Auk* 108, 619–637.
- McDonald, D.B., 1993. Delayed plumage maturation and orderly queues for status: A manakin mannequin experiment. *Ethology* 94, 31–45.
- Mougeot, F., Dawson, A., Redpath, S.M., Leckie, F., 2005. Testosterone and autumn territorial behavior in male red grouse *Lagopus lagopus scoticus*. *Horm. Behav.* 47, 576–584.
- Mougeot, F., Martinez-Padilla, J., Webster, L.M.I., Blount, J.D., Perez-Rodriguez, L., Pieltney, S.B., 2009. Honest sexual signalling mediated by parasite and testosterone effects on oxidative balance. *Proc. R. Soc. Lond. B* 276, 1093–1100.
- Peters, A., Asstheimer, L.B., Cockburn, A., 2001. The annual testosterone profile in cooperatively breeding superb fairy-wrens, *Malurus cyaneus*, reflects their extreme infidelity. *Behav. Ecol. Sociobiol.* 50, 519–527.
- Poiani, A., Fletcher, T., 1994. Plasma levels of androgens and gonadal development of breeders and helpers in the bell miner (*Manorina melanophrys*). *Behav. Ecol. Sociobiol.* 34, 31–41.
- Prum, R.O., 1994. Phylogenetic analysis of the evolution of alternative social behavior in the manakins (aves: Pipridae). *Evolution* 48, 1657–1675.
- Reyer, H.R., Dittami, J.P., Hall, M.R., 1986. Avian helpers at the nest: Are they psychologically castrated? *Ethology* 71, 216–228.
- Schlinger, B.A., Day, L.B., Fusani, L., 2008. Behavior, natural history and neuroendocrinology of a tropical bird. *Gen. Comp. Endocrinol.* 157, 254–258.
- Schmidt, L.G., Bradshaw, S.D., Follett, B.K., 1991. Plasma levels of luteinizing hormone and androgens in relation to age and breeding status among cooperatively breeding Australian magpies (*Gymnorhina tibicen latham*). *Gen. Comp. Endocrinol.* 83, 48–55.
- Schoech, S.J., Mumme, R.L., Moore, M.C., 1991. Reproductive endocrinology and mechanisms of breeding inhibition in cooperatively breeding Florida scrub jays (*Aphelocoma c. coerulescens*). *Condor* 93, 354–364.
- Schoech, S.J., Mumme, R.L., Wingfield, J.C., 1996. Delayed breeding in the cooperatively breeding Florida scrub-jay (*Aphelocoma coerulescens*): inhibition or the absence of stimulation? *Behav. Ecol. Sociobiol.* 39, 77–90.
- Schoech, S.J., Reynolds, S.J., Boughton, R.K., 2004. Endocrinology. In: Koenig, W.D., Dickinson, J.L. (Eds.), Ecology and evolution of cooperative breeding in birds. Cambridge University Press, Cambridge, pp. 128–141.
- Sick, H., 1967. Courtship behavior in manakins (Pipridae): a review. *Living Bird* 6, 5–22.
- Soares, M.C., Bshary, R., Fusani, L., Goymann, W., Hau, M., Hirschenhauser, K., Oliveira, R. F., 2010. Hormonal mechanisms of cooperative behaviour. *Philos. Trans. R. Soc. B Biol. Sci.* 365, 2737–2750.
- Stacey, P.B., Koenig, W.D. (Eds.), 1990. Cooperative breeding in birds. Long term studies of ecology and behavior. Cambridge University Press, New York.
- Van Hout, A.J.M., Eens, M., Darras, V.M., Pinxten, R., 2010. Acute stress induces a rapid increase of testosterone in a songbird: implications for plasma testosterone sampling. *Gen. Comp. Endocrinol.* 168, 505–510.
- Vleck, C.M., Brown, J.L., 1999. Testosterone and social and reproductive behaviour in aphelocoma jays. *Anim. Behav.* 58, 943–951.
- Wikelski, M., Hau, M., Wingfield, J.C., 1999. Social instability increases plasma testosterone in a year-round territorial neotropical bird. *Proc. R. Soc. Lond. B* 266, 551–556.
- Wikelski, M., Hau, M., Robinson, W.D., Wingfield, J.C., 2003. Reproductive seasonality of seven neotropical passerine species. *Condor* 105, 683–695.
- Wingfield, J.C., Hegner, R.E., Dufty, A.M., Ball, G.F., 1990. The challenge hypothesis – theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am. Nat.* 136, 829–846.
- Wingfield, J.C., Hegner, R.E., Lewis, D.M., 1991. Circulating levels of luteinizing hormone and steroid hormones in relation to social status in the cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali*. *J. Zool.* 225, 43–58.