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Testosterone and its effects on courtship in golden-collared manakins (*Manacus vitellinus*): Seasonal, sex, and age differences

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Abstract

Male golden-collared manakins gather on leks and perform an acrobatic display to attract females. In temperate breeding species, testosterone (T) activation of courtship displays has been well studied. Few studies have examined T activation of displays in tropical species; even fewer have explored the activational role of T in elaborate courtship displays such as in the manakin. In some tropical species, including manakins, territorial aggression or song behavior are uncoupled from T. We have previously shown that T activates display behavior in manakin males when endogenous T levels are low in the non-courtship season. To understand how T functions in breeding birds, we examined T levels in a large group of manakins sampled during the courtship and non-courtship season. In addition, during the courtship season, we gave T implants to adult males, juvenile males, and females. We found that T levels were low during the non-courtship season and comparatively higher on average during the courtship season. However, T levels were low in many adult males during the courtship season, especially when compared to temperate breeding species. Regardless of initial endogenous T levels during the courtship season, T implants did not increase the display frequency of adult males. T-treated females and juvenile males did display under similar conditions. Our data suggest that the effects of T on manakin display vary with season, sex, and age and that high T is not necessary for display.

Keywords: Testosterone; Courtship; Tropical; Manakins; Seasonal; Display

The understanding of how testosterone (T) activates reproductive behaviors in birds, such as territoriality and song, draws largely from experiments on species breeding in the north temperate zone. Males of these species show discrete seasonality in both T levels and reproduction and a direct link has been shown between T and the activation of display behaviors (Balthazart, 1983; Silver and Ball, 1989; Wingfield et al., 1999). Several recent studies have examined birds breeding in the tropics to see if, like their temperate breeding counterparts, T is elevated during the breeding season to activate reproductive and aggressive behaviors (Dittami, 1986, 1987; Fedy and Stutchbury, 2006; Goymann et al., 2004; Hau et al., 2000; Moore et al., 2004a,b; Wikelski et al., 1999, 2003).

The results of these studies reveal unique patterns of sex steroid secretion and the hormonal control of behavior. In comparison to temperate breeding species, T levels of tropical male birds are generally lower year round and breeding seasons are longer (Dittami, 1986, 1987, Dittami and Gwinner, 1990; Wikelski et al., 2003). Although some degree of seasonality in reproduction appears to be the rule (Dittami, 1986, 1987; Dittami and Gwinner, 1990; Wikelski et al., 2003), there appears to be little association between T levels and territoriality (Fedy and Stutchbury, 2006; Wikelski et al., 2003). Some species are highly territorial year round despite low T levels and other species have variable T levels with consistent levels of territoriality (Wikelski et al., 2003). Experiments testing the activational effects of T have produced conflicting results. In

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some cases, T appears to influence song and aggressive behavior (tropical spotted antbird *Hylophylax n. naevioides*) (Hau et al., 2000). In other cases, T does not appear to affect territorial aggression such as in tropical rufous-collared sparrows (*Zonotrichia capensis*) (Moore et al., 2004a), white-browed sparrow weavers (*Plocepasser mahali*), bay wrens (*Thryothorus nigricapillus*) (Levin and Wingfield, 1992), and white-bellied antbirds (*Myrmeciza longipes*) (Fedy and Stutchbury, 2006). Consequently, predictions about the role of T in tropical bird behavior are not straightforward (Goymann et al., 2004).

In addition to song and territorial behavior, many male birds perform complex courtship displays. In temperate breeding species, courtship behaviors are dependent on sex steroids (Balthazart, 1983), but few studies have investigated this hypothesis in tropical species (but see Chastel et al., 2005). Because such studies stand to provide general insight into the neuroendocrinology of behavior in tropical animals, we have examined the role of hormones in activating the acrobatic courtship display of golden-collared manakins (*Manacus vitellinus*) of Panama. Golden-collared manakins collect in aggregates of 5–20 males (leks) and perform a highly acrobatic dance (Chapman, 1935; Schlinger et al., 2001) punctuated by loud "snapping" sounds apparently made with the wings. Males begin to establish courtship arenas in January (Fusani et al., in press) that they occupy until June or July. Juvenile males and adult males without arenas display at reduced rates throughout the year. In a previous study, we found that exogenous T increased the display behavior of all males during the non-courtship season (Day et al., 2006). Before treatment and in untreated birds, circulating T levels were nearly undetectable (Day et al., 2006); presumably the reason courtship behavior is minimal. Two previous studies examined seasonal changes in T levels in golden-collared manakins (Fusani et al., in press; Wikelski et al., 2003). These studies showed that T levels are low throughout the courtship season except for what appear to be modest peaks in plasma T either during lek formation (Fusani et al., in press) or when mating begins (Wikelski et al., 2003). Although T levels appear to fluctuate during the courtship season, display behavior (Fusani et al., in press) and territoriality (Wikelski et al., 2003) do not change, suggesting a lack of congruence between courtship and territoriality, and circulating T.

To better understand the relationship between T and behavior in manakins, we measured plasma T in a large number of individuals during the courtship season and in the non-courtship season. We found that circulating T varies seasonally only in adult males with all non-courting birds having low levels of plasma T. In general, adult males had elevated levels of plasma T in the courtship season, but the levels varied widely; some males had undetectable amounts of T in blood whereas others had levels as high as many north temperate zone breeding male birds. Females and juvenile males generally had low levels of T year round. To determine how exogenous T might influence display behavior of birds during the courtship season, adult males, females, and juvenile males were treated with exogenous T and the frequency of display behavior was measured.

Methods

All birds were captured in forest near Gamboa, Panama, with permission of local authorities and under the guidelines of the Animal Care and Use Committee of UCLA. We collected blood samples and implanted birds during the courtship season (February–April) when females visit arenas and mating takes place and collected blood samples in the middle of the non-courtship season (July–September) when males' display much less frequently, fewer arenas are found on leks and arenas are not dominated by a single male.

Animals and treatment

All birds were captured in mistnets typically between 0700 and 0800 and the approximate time in the net was recorded. Birds were bled by venipuncture within 10 min of removal from the net and color-banded. Treated birds were implanted subcutaneously with 60-day release/1.5 mg T pellets or matching inert pellets (Innovative Research of America, Sarasota, FL). These T-filled pellets effectively elevate plasma T levels and activate courtship display in males during the non-courtship season (Day et al., 2006). Field observed birds were implanted and released. Captive birds were given implants 1–3 days after capture and bled at several time points during captivity as described below.

To study seasonal variation in T levels, blood was drawn from individuals captured randomly from five different leks during the courtship season (13 March to 7 April) and non-courtship season (29 July to 20 September). We collected samples from 9 green plumaged individuals (2 juvenile males and 7 females, see sexing below) and 14 adult males during the courtship season. During the non-courtship season, we collected samples from 22 green plumaged individuals (10 males and 12 females) and 18 adult males.

For field observations of T effects on courtship behavior, 16 adult males were randomly assigned to the T ($n=8$) or Control groups ($n=8$). Birds were captured in February and March from four leks that were independent from those used in other studies. Each lek had an equal number of Control or T-treated birds (1–3).

Sixteen other birds (three females, five juvenile males, and eight adult males) were captured from five leks in March and April to examine the effects of T implants on different age/sex groups in captivity. All juveniles, females, and four adult males were assigned to T treatment and four adult males were assigned to a Control group. The individuals used are unique to this experiment but some of the leks used overlap with those used for studies of seasonal variation in T levels. Birds were taken into captivity and housed in a well-lit and ventilated room at the STRI facilities in Gamboa in cages approximately 360×290 mm (for details, see Day et al., 2006). Natural light was supplemented by fluorescent lighting for 12 h. Food and water were available *ad libitum*.

Sexing birds

The sex of green-plumaged birds cannot be determined by external morphology. Therefore, birds were sexed genetically using Chelex (Chelex 100 resin, Sigma) to isolate DNA from packed red blood cells (Jensen et al., 2003; Walsh et al., 1991) and primers (forward primer, 5′-YTKCCAAGRAT-GAGAAACTG-3′ and reverse primer 5′-TCTGCATCACTAAAKCCTTT-3′; Agate et al., 2002) to amplify Z and W isoforms of the CHD-1 gene and introns spanned by this gene. Polymerase chain reaction amplification and thermal profiles are detailed in Day et al. (2006). Individuals of known sex were incorporated into PCR runs to validate the accuracy of the reaction.

Hormone assay

RIA procedures used have been validated for use with zebra finch (*Taeniopygia guttata*) and manakin plasma (Day et al., 2006; Schlinger et al., 1999) and described in detail elsewhere (Day et al., 2006). Briefly, we used a Coat-A-Count kit (Diagnostic Products Corporation, Los Angeles, CA) to measure total T. Where possible, we used 50-μl duplicate samples for each individual. In addition to manakin samples, we included human 1 ng/ml samples in triplicate.

We performed two separate assays for the study of seasonal variation in T levels and for samples taken from birds observed in captivity. For the cross-

season study, the intra-assay CV for the duplicates that were above the minimum detection level was 7.4% ($n=2$) and for the three human 1 ng/ml standards the coefficient of variation (CV) was 5.3%. The mean of the human 1 ng/ml standards was 0.97 ng/ml. The minimum detection level was 0.021 ng/ml.

For captive animals, we could not accurately calculate the intra-assay CV as only one duplicated manakin sample was above the minimum detection limit. The CV for this sample was 13%. The CV for the three human 1 ng/ml samples was 8.0% and the mean for these samples was 1.2 ng/ml. The upper and lower limits of detection were determined to be 0.01 ng/ml and 16 ng/ml, respectively.

Field observations

Between February 21 and March 13, we observed implanted males for 30-min sessions from the 2nd to the 23rd day after implantation, performing at least 2 observations/bird for each 3-day period. Birds were observed between 0700 and 0900 and between 1230 and 1530, when activity typically peaks within the leks. The observer sat about 10 m from the arena. From this position the observer had a clear view of the arena but seemed to be ignored by the displaying males. Nevertheless, behavioral recording began after a 15-min acclimation period. We recorded the number of times the following previously described behaviors were performed (Chapman, 1935; Day et al., 2006; Schlinger et al., 2001): (1) jump-snap displays, hopping from one vertical twig to another across a cleared display arena while wingsnapping in mid-flight; (2) wingsnaps, snaps of the wings produced during the jump-snap display; (3) rollsnaps, a series of quick loud snaps; and (4) the number of cheepoos calls, a distinct call usually produced after rollsnaps.

Some birds were not seen during the first few days of observation, so we performed analyses for all birds from the fifth day after implant. Two control birds did not have arenas on the experimental leks and were therefore excluded from the experiment. Daily totals for each behavior measured were summed and the number of behaviors per minute was calculated for each bird.

Captive observations

T-treated and control adult males were implanted in matched pairs, observed for 7–10 days, and bled at the end of this period. At the end of 10 days, adult males had performed almost no display behavior, so the four control males were transferred to a separate experiment not discussed in this paper. We continued to observe the remaining T-treated males along with T-treated females and juvenile males.

As we did not know the sex of green plumaged birds when implanted, we were unable to match implant days across sex/age groups as we had done with adult males. This meant that at the end of the experiment the groups did not have equivalent number of days implanted. To minimize differences in the number of days implanted between groups, we analyzed behavior of birds up to day 17 post-implant. The resultant average days implanted was 17 for adult males, 16 for juvenile males, and 16.3 for females. As a group these birds had been implanted an average of 18 days (SE ±1.09) prior to the end of the experiment.

A blood sample was drawn at 10 days post-implant for all groups. In addition, a final blood sample was taken when the experiment ended. All birds were then released at the original site of capture.

Behavior was recorded daily for 2 h starting around 0630 using a Sony CCD-TRV68 video camera with all cages visible in the recording frame. From these recordings, we counted the number of wingsnaps, rollsnaps, and snips (distinctly low amplitude snaps produced with little obvious movement of the wings or by obvious movement of the tail). Calls were not counted as it was difficult to discern which individual had made the call. For analyses, all behaviors were expressed per min the individual was filmed.

Statistical analyses

All analyses were performed using Statview 5.0 (SAS Inst. Inc.) with significance set at $\alpha=0.05$. In order to compare differences across sex/age groups in T levels and behavior, we used Kruskal–Wallis test followed by Mann–Whitney *U*-tests. To examine T levels between seasons we used a Mann–Whitney *U*-test. To compare behavior of Controls and T-treated birds, we used *T*-tests for field observed birds but used Mann–Whitney *U*-tests for captive birds as subject numbers were lower. Wilcoxon signed ranks tests were used to examine differences in plasma T levels of captive birds for blood drawn in the field, at 10 days, and at 18 days (end of the experiment). Spearman rank correlations were used to examine relationships between behavior and T levels in captive birds.

Results

Seasonal variation in T levels

T levels were highly variable for courting adult males showing a continuum of values from non-detectable to 6 ng/ml. However, most (9/14) of the adult courting males examined had levels of T that were low, less than 0.38 ng/ml (there was a natural break at this level, see Fig. 1). Assuming these samples accurately reflect hormone levels in the overall population, we can assume that more than half of the adult males have low T levels during the courtship season.

Courting juvenile males had less than 0.89 ng/ml T. No female had higher than 0.2 ng/ml T in either the courtship or non-courtship season (see Fig. 1). For adult males, levels of plasma T for birds sampled during the courtship season were higher than those for birds sampled in the non-courtship season ($U=4$, $p<0.0002$) whereas there was no difference in T levels between seasons for juvenile males and females (see Fig. 1). There was a significant difference between the three groups during the courtship season ($H=14.18$, $p<0.0008$) with females having significantly lower T levels than juvenile males ($U=1$, $p<0.04$) and adult males ($U=0$, $p<0.0002$). Adult courtship season males did not differ significantly from juvenile courtship season males. There was no difference between the groups during the non-courtship season. There

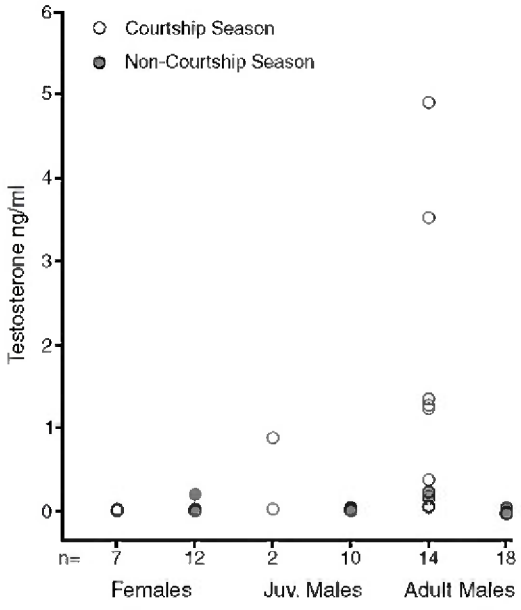

Fig. 1. Sex/age and season field levels of plasma T. Courtship season (March 13 to April 7) and non-courtship season (July 29 to September 20) for golden-collared manakins. Numbers on *X* axis indicate subject numbers.

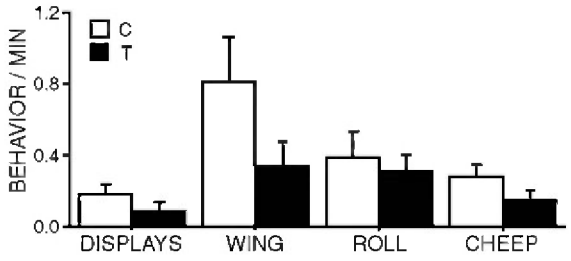

Fig. 2. Average display behaviors per min of field observation time for adult courting males given either a T pellet (T) or an inert pellet (C). Shown are jump-snap displays (displays), wingsnaps (wing), rollsnaps (rolls), and cheepoo calls (cheep). No differences were significant.

was no relationship between T levels and the time of day the sample was taken or the time the bird was in the net for any groups.

Field implantation experiment

Males were actively displaying on arenas at all the lek sites used in this study. As has been described previously for manakins (Stein and Uy, 2006), some males displayed vigorously whereas others showed comparatively low levels of display (control, mean=0.18, range=0.05–0.39/min; T treated, mean=0.09 range 0–0.27/min). We predicted that exogenous T would increase display rate of the relatively inactive males. This expectation was not supported. Implant condition had no effect on behavior (Fig. 2). None of the components of the display (jump-snap displays $t(12)=1.4$, $p=0.18$; wingsnaps $t(12)=1.83$, $p=0.09$; rollsnaps $t(12)=0.46$, $p=0.65$; cheepoos $t(12)=1.68$, $p=0.12$) were higher in T-implanted birds.

Captive implantation experiment

Testosterone levels

Upon capture, there was no significant difference between T levels of adult male birds assigned to control and treatment conditions (see Fig. 3B). As expected, field sampled levels of plasma T differed between sex/age groups ($H=6.78$, $p<0.03$) as a result of significantly lower levels of T in females compared to both adult males ($U=1$, $p<0.04$) and juvenile males ($U=0$, $p<0.03$, Fig. 4B).

After treatment, T levels were significantly lower in adult males given inert pellets than in those given T pellets ($U=0$, $p<0.02$, see Fig. 3B). There were no differences between sex/age groups in T levels either at post-implant day 10 or day 18, suggesting that T implants were effective in equating groups (Fig. 4B). Furthermore, across groups there was an increase from field levels of T at post-implant day 10 ($W=67$, $p<0.03$) but levels decreased from post-implant day 10 to post-implant day 18 ($W=78$, $p<0.002$, Fig. 4B). This result suggests that the T implants did not give consistent circulating T levels over the 60 days of release. Nevertheless, and more importantly, the T pellets did maintain levels at or above physiological levels for the duration of the experiment.

Behavioral observations: adult males

Surprisingly, display behavior of both adult male groups was extremely low compared to field levels and compared to the extent of the display that we have previously seen in captivity. Most adult birds actively display in the field at this time of the year even if at low levels. Previous experiments had shown that non-breeding juvenile birds perform wingsnaps, and rollsnaps reliably in captivity and, to the extent possible in the confines of the cage, also perform jump-snap displays (Day et al., 2006).

As in the field study, there was no significant difference between T-treated and control birds for any of the display elements measured (all $p>0.56$, see Fig. 3A). Neither treatment group performed any rollsnaps (see Fig. 3A). The lack of behavioral change in the T-treated group compared to the controls was irrespective of the initial endogenous concentration of circulating T. Adult males with initial T levels similar to that of males in the non-courtship season (<0.38 ng/ml) had similar rates of display to males with initially high endogenous T-levels (>1 ng/ml). This is interesting because the same T pellets increase display activity when given to adult males with similar endogenous T levels during the non-courtship season (Day et al., 2006). Importantly, there was no correlation between T levels pre or post-implant and any behavioral variables (all $p>0.12$) indicating that birds with low T levels are not necessarily those birds performing display elements at low levels.

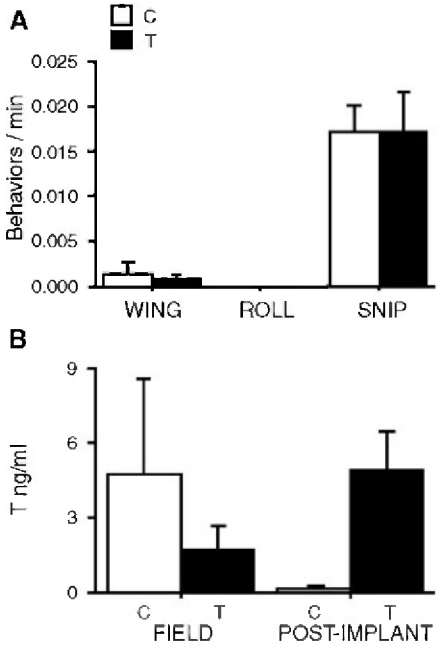

Fig. 3. (A) Average courtship behaviors per min of filming in captivity for adult courting males given either a T pellet (T) or an inert pellet (C). Shown are wingsnaps (wing), rollsnaps (roll), and snips. No differences were significant. (B) Levels of plasma T in the field prior to implant and post-implant for T and C birds. Field levels of T were not significantly different between groups, T-treated individuals had higher T post-implant.

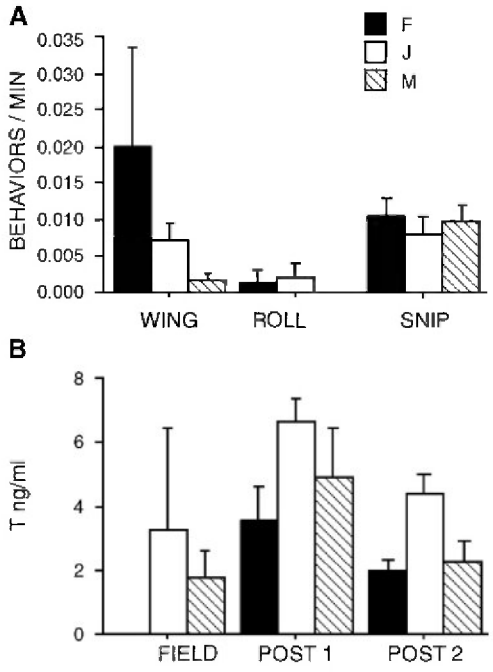

Fig. 4. (A) Average courtship behaviors per min of filming in captivity for courtship season adult males (M), females (F), and juvenile (J) males given T pellets. Shown are wingsnaps (wing), rollsnaps (roll), and snips. Groups differed in wingsnaps and rollsnaps with juvenile males performing significantly more wingsnaps and rollsnaps than adult males. (B) Levels of plasma T in the field prior to implant and at post-implant day 10 (Post 1) and at the end of the experiment (Post 2).

Behavioral observations: sex/age differences

Although T-treated adult males rarely displayed, T-treated juvenile males and females did. We found behavioral differences between sex/age groups in some elements of the courtship display. There was a significant difference in wingsnaps ($H=5$, $p<0.05$) and rollsnaps ($H=7.07$, $p<0.03$) across the three T-treated groups. Mann–Whitney U paired comparisons show that juvenile males produced more wingsnaps ($U=1$, $p<0.02$) and rollsnaps ($U=0$, $p<0.01$) than adult males. The mean number of wingsnaps and rollsnaps produced by adult females was marginally higher than adult males ($p=0.07$ for both). Juvenile males and females produced similar numbers of rollsnaps and wingsnaps. There were no differences between groups in the number of snips (Fig. 4A). There was no correlation between T levels measured either at day 10 or at day 18 and any of the behaviors measured for any of the sex/age groups.

Because we did not include untreated juvenile males and females in the present experiment, we could not conclude that the display behavior measured was activated by exogenous T. Therefore, we performed an additional set of observations one year later on 14 untreated green-plumaged manakins ($n=8$ females, $n=6$ males). Birds were audio recorded simultaneously 30–90 min each day for 7 days (average daily total of 392 min recording) in cages about one-third the size of that used in the previous study. Birds in this group performed an average of 0.002±0.0008 wingsnaps/min, 0.0021±0.001 snips/min, and no roll snaps. This was far less than the group average for our T-treated green plumaged birds reported above, 0.012±0.014 wingsnaps/min, 0.009±0.006 snips/min, 0.001±0.002 rollsnaps/min. Following audio recording, four males and one female were moved into the larger cages used in the experiment reported above. These birds were videotaped for 40 –157 min daily for 7 days for an average of 399 min/bird total filming time. These untreated males and the one female performed no wingsnaps or roll snaps during this observation period (compared to results of the T-treated birds from the previous year: wingsnaps (males 0.008±0.002, females 0.02±0.01) and rollsnaps (males 0.003±0.001, females 0.002±001)). The difference between the five T-treated males and four untreated males was significant for both wingsnaps ($U=0$, $p<0.01$) and rollsnaps ($U=0$, $p<0.01$). Differences in numbers of snips between untreated (0.002±0.001) and T-treated (0.008±0.003) males were not significant and these group averages were similar to the performances for females (untreated 0.002 snip/min, T treated 0.01±0.002). In addition, females are not known to display in the wild and the courtship behavior of both females and juvenile males slowly increased and then decreased (Fig. 5) corresponding with changes in T levels (Fig. 4). In contrast, courtship behavior of adult males remained unchanged under the same changing hormonal environment (Fig. 5). These data strongly support the conclusion that, unlike adult males, T activates courtship in captive females and juvenile males during the courtship season.

Interestingly, in a previous study (Day et al., 2006), we found that non-courtship season, T-treated juvenile males performed complete jump-snap displays in captivity (they quickly hopped from perch to perch while wingsnapping). In the current experiment, no birds performed any typical jump-snap displays even after T treatment. Rather, wingsnaps were typically separated by several seconds or minutes and when performed fairly quickly in succession were not accompanied by hops or acrobatic movements (with the exception of a single wingsnap by one adult male). This suggests that T induces a more complete courtship display during the non-breeding season than during the breeding season.

Discussion

Our data indicate that, like many north temperate zone breeding species (Wingfield et al., 1990), T varies seasonally in adult male golden-collared manakins but not in juvenile males

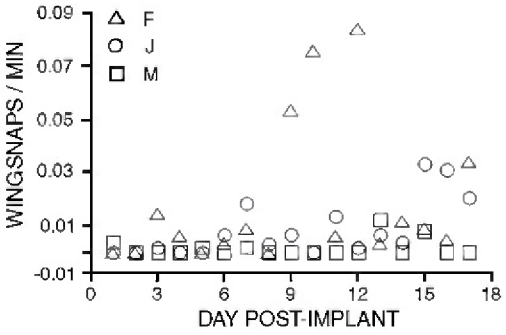

Fig. 5. Wingsnaps per min of time filmed in captivity for adult males (M), females (F), and juvenile (J) males given T pellets as a function of days post-implant.

and females and that T levels during the courtship season are higher in adult males than in females. These data agree with previous reports suggesting T is related to seasonal activation of display. Notably, however, T levels in males captured in the midst of the courtship season were highly variable with approximately 65% of males having low (<0.38 ng) or undetectable levels of T. These results suggest that high levels of T are not necessary to maintain courtship behavior during the long courtship season. These data also raise the possibility that exogenous T could increase courtship in those males with low T levels as it does in males with low T during the non-courtship season. Unexpectedly, we found that T implants did not increase the display behavior of courting adult males in the field or in captivity regardless of initial T levels. By contrast, juvenile males and females did display after T implantation under the same conditions. Finally, individual T levels never correlated with levels of courtship display. It remains possible that T levels may contribute to details of the acrobatic movements of the display or to overall reproductive success. Additional experiments are currently underway to address these possibilities.

Testosterone and courtship display in adult male manakins

A previous study from our lab suggested that T levels are modestly elevated (approximately 1 ng/ml) in adult male manakins when leks are first established (in late January). Three weeks later when the same birds were recaptured and bled T levels had declined to near basal levels (Fusani et al., submitted in press). Although T levels declined, display behavior in these males was high and unchanging (Fusani et al., in press). We have also shown previously that during the non-breeding season T levels in males are low and exogenous T can activate display behavior (Day et al., 2006). From these observations, we hypothesized that the gonads likely begin secreting T in late January thus starting the breeding season and activating courtship behavior. We hypothesized that after this initial activation, T becomes uncoupled from behavior as the courtship season progresses (Day et al., 2006; Fusani et al., in press). The results of the current studies are consistent with this hypothesis in that increased T does not alter the measured display components in the middle of the breeding season. However, these data also reveal greater seasonal changes at the population level, more individual variability, and higher levels of T mid-courtship season in some individuals than previously found in our studies. The mechanisms underlying the apparent uncoupling of T levels from behavior thus appear complex and must be explained.

T levels may be uncoupled from display intensity because after initial T activation of display, adult males become insensitive to later increases in T as was suggested previously in a study of ring doves (Fusani and Hutchison, 2003). In that experiment, however, exogenous T probably had no behavioral effects because endogenous T levels were already sufficiently high (i.e., at or above a threshold level) to activate courtship behavior at individual maximums (Fusani and Hutchison, 2003). In many male manakins, however, T levels are low, in some cases similar to levels of non-courting males during the non-courtship season (Day et al., 2006). Perhaps, in manakins, neural circuits underlying the performance of courtship behavior become unusually sensitive to very low circulating T levels during the middle of the courtship season. Increases in neural sex steroid receptors or changes in steroid-metabolic enzymes might underlie such changes in sensitivity (Canoine et al., in press). Future studies can address this possibility.

Although T is low in many individuals, levels are quite variable across individuals and time, with levels quite high in some adult males. The purpose of these high T levels is unknown but may stem from rapid changes in behavioral dynamics of individual leks. For example, the arrival of a female at or near a male's arena, unsettled male–male competition for arena space, or changes in the overall social network of a lek by the arrival of other males could increase or decrease T levels in different individuals. It is known that T is elevated in the tropical spotted antbird in reaction to experimental territorial intrusions (Wikelski et al., 1999). A similar test of the "challenge hypothesis" (Wingfield et al., 1990) in manakins might reveal rapid changes in levels of plasma T.

Although such experiments might help determine why T is variable across time, they would not explain why courtship behavior is not correlated with T levels nor how courtship behavior is maintained when T levels are similar to those found in non-breeding males. Perhaps, in manakins, courtship traits are simply not proportionally related to T levels as has been found for some species (Adkins-Regan, 2005; Fusani and Hutchison, 2003; Hews and Moore, 1997; see Borgia and Wingfield, 1991; Foerster et al., 2002 for species that show T/display correlation). However, the display of full courtship behavior when T levels are quite low might suggest alternative mechanisms for courtship maintenance such as other steroid hormones or non-gonadal steroids like dehydroepiandrosterone (DHEA) as precursors for other sex steroids. DHEA, produced by the adrenals and possibly synthesized in the brain itself, is correlated with levels of aggressive vocalizations in male and female spotted antbirds (Hau et al., 2004). It will be interesting to see whether other sexual traits that in temperate species depend on seasonal increases in T levels, such as spermatogenesis, are also maintained in manakins with very low gonadal T.

Behavior of females and juvenile males

Our results suggest that T-treated juvenile males will display courtship behaviors in captivity during the courtship season as we have previously shown for juvenile males in the field and captivity during the non-courtship season (Day et al., 2006). It is interesting to note that levels of display seen in this experiment were less than half of the display rates found in juvenile males implanted during the non-courtship season. This suggests seasonal differences in the ability of T to increase display elements similar to the seasonal differences seen in T effects in adult males.

The behavior of females has not been given the attention it deserves, in part because females and juvenile males cannot be distinguished in the field. It has generally been thought that females rarely produce courtship display elements. Our results

show that T-treated females display as much as juvenile males. As reported above, during many hours of audio and video recording of several captive females during the courtship season, no wingsnaps or rollsnaps and only a few snips were detected. In a previous study (Day et al., 2006), we filmed two untreated females during the non-courtship season for 147 h and saw no rollsnaps, a single wingsnap, and ten snips. This was the first documented evidence that females could produce "snaps". In the present study, over 108 h of filming, three females produced 111 wingsnaps, 8 rollsnaps, and 62 snips clearly demonstrating that females can perform these masculine courtship behaviors when given T implants. These results suggest that neural circuits underlying the expression of at least some of the male courtship behaviors are not organized differently during development in males and females, as are copulatory behaviors in many vertebrate species (Breedlove, 1992; Morris et al., 2004) and as is courtship song in the zebra finch (*T. guttata*) (Schlinger, 1998). Rather they suggest that the expression of masculine courtship is dependent solely upon the activational effects of adult T, much like T can activate masculine copulation and courtship in females of some species (Balthazart and Ball, 1995) and song in female canaries (*Serinus canaries*) and budgerigars (*Melopsittacus undulates*) (Nottebohm, 1980, Nespor et al., 1996). In this case, it seems that masculine courtship behaviors are not seen in female manakins because they are not normally exposed to sufficient circulating T.

Further, it suggests that sex differences in manakin muscle anatomy and physiology (Schultz et al., 2001) and in spinal cord T accumulation (Schultz and Schlinger, 1999) are also created by sex differences in circulating T in adults. Courtship of wild males is far more complex (Schlinger et al., 2001) than the discrete display elements that we observe in any of the birds in captivity. We do not know if females treated with T would be able to choose a proper site and then create a courtship arena, as do males, or perform the elaborate flight moves and acrobatics of the adult males. It is intriguing to consider that T could activate this full masculine behavioral repertoire in females or whether some behaviors are established uniquely in males. Future experiments implanting T into wild females, or into females occupying large forest aviaries are required to examine these ideas.

We do not know why T activates courtship behavior in juvenile males and females but not in adult males during the courtship season. Remembering that we know that T activates courtship in adult males during the non-courtship season (Day et al., 2006), it leads us to hypothesize that once T activates courtship initially, it is no longer required for the duration of the courtship season. Perhaps only after T declines for an extended period and social (e.g., lack of females) or environmental (e.g., increased rain) conditions reduce courtship in males will T again be required to reactivate courtship for a new season of activity. This explains why T treatments had no impact on courtship in adult male manakins during the courtship season. Because juvenile males and females see little or no endogenous T, then exogenous T can activate courtship at any time of the year. It is also possible that compared to females and juvenile males, adult males have a stronger stress response to captivity resulting in an increase in corticosterone that could blunt the effects of T. Similarly, adults may be subject to the blunting effects of corticosterone because the outcome of aggressive interactions has altered levels of corticosterone, testosterone, or other interacting neurochemicals. Such effects are known to occur in species as diverse as fish, lizards, birds, and primates (Knapp and Moore, 1995, 1996, 1997; Sapolsky, 1989; Summers, 2002). Just as some social constraints may inhibit display, we also believe that some social conditions not present in captivity, such as the presence of a female near the courtship arena, may be required for adult males to perform complete displays. Social constraints may not operate so strongly in juvenile males and females. Each of these hypotheses require additional experiments to determine what maintains courtship in the absence of T, why T levels are so variable in adult males, why T activates display in females and juvenile males but not adults, and to examine whether T can organize neural circuits controlling behaviors each year with seasonal, not life long, sexually dimorphic characteristics.

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References

Adkins-Regan, E., 2005. Hormones and Animal Social Behavior. Princeton University Press, Princeton.

Agate, R.J., Perlman, W.R., Arnold, A.P., 2002. Cloning and expression of zebra finch (*Taeniopygia guttata*) steroidogenic factor 1: overlap with hypothalamic by not with telencephalic aromatase. Bio. Reprod. 66, 289–295.

Balthazart, J., 1983. In: Farner, D.S., King, J.R., Parker, K.C. (Eds.), Hormonal Correlates of Behavior. Avian Biol., vol. VII. Academic Press, San Diego, pp. 221–365.

Balthazart, J., Ball, G.F., 1995. Sexual differentiation of brain and behavior in birds. Trends Endocrinol. Metab. 6, 21–29.

Borgia, G., Wingfield, J.C., 1991. Hormonal Correlates of Bower Decoration and Sexual Display in the Satin Bowerbird (*Ptilonorhynchus violaceus*).

Breedlove, S.M., 1992. Sexual differentiation of the brain and behavior. In: Becker, J.B., Breedlove, S.M., Crews, D. (Eds.), Behavioral Endocrinology. MIT Press, Cambridge, Mass, pp. 53–68. Vol.

Canoine, V., Fusani, L., Schlinger, B., Hau, M., in press. Low sex steroids, high steroid receptors: increasing the sensitivity of the non-reproductive brain. J. Neurobiol.

Chapman, F.M., 1935. The courtship of Gould's manakin (*Manacus vitellinus vitellinus*) on Barro Colorado Island, canal zone. Bull. Am. Mus. Nat. Hist. 471–525.

Chastel, O., Barbraud, C., Weimerskirch, H., Lormée, H., Lacroix, A., Tostain, O., 2005. High levels of LH and testosterone in a tropical seabird with and elaborate courtship display. Gen. Comp. Endocrinol. 140, 33–40.

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.

Dittami, J.P., 1986. Seasonal reproduction, moult and their endocrine correlates in two tropical *Ploceidae* species. Journal of Comparative Physiology B 156, 641–647.

Dittami, J.P., 1987. A comparison of breeding and moult cycles and life histories in two tropical starling species: the blue-eared glossy starling *Lamprotornis chalybaeus* and Rüppell's long-tailed glossy starling *L. purpuropterus*. Ibis 129, 69–85.

Dittami, J. P., Gwinner, E., 1990. Endocrine correlates of seasonal reproduction and territorial behavior in some tropical passerines. In: M. Wada, S. Ishii, C. G. Scanes (Eds.), Endocrinology of Birds: Molecular to Behavioral., Vol. Japan Scientific Society Press, Springer-Verlag, Berlin, Tokyo, Berlin, pp. 225–233.

Fedy, B.C., Stutchbury, B.J.M., 2006. Testosterone does not increase in response to conspecific challenges in the white-bellied antbird (*Myrmeciza longipes*), a resident tropical passerine. The Auk 123 (1), 61–66.

Foerster, K., Poesel, A., Kunc, H., Kempenaers, B., 2002. The natural plasma testosterone profile of male blue tits during the breeding season and its relation to song output. J. Avian Biol. 33, 269–275.

Fusani, L., Day, L. B., Canoine, V., Reinemann, D., Hernandez, E., Schlinger, B. A., in press. Androgen and the elaborate courtship behavior of a tropical bird. Horm. Behav.

Fusani, L., Hutchison, J.B., 2003. Lack of changes in the courtship behavior of male ring doves after testosterone treatment. Ethol. Ecol. Evol. 15 (2), 143–157.

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 (3), 327–334.

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

Hau, M., Stoddard, S.T., Soma, K.K., 2004. Territorial aggression and hormones during the non-breeding season in a tropical bird. Horm. Behav. 45, 40–49.

Hews, D.K., Moore, M.C., 1997. Hormones and sex-specific traits: critical questions. In: Beckage, N.E. (Ed.), Parasites and Pathogens: Effects of Host Hormones and Behavior. Chapman and Hall, New York, pp. 277–292.

Jensen, T., Pernasetti, F.M., Durrant, B., 2003. Conditions for rapid sex determination in 47 avian species by PCR of genomic DNA from blood, shell-membrane blood vessels, and feathers. Zoo Biol. 22, 561–571.

Levin, R.N., Wingfield, J.C., 1992. The hormonal control of territorial aggression in tropical birds. Ornis Scand. 23 (3), 284–291.

Knapp, R., Moore, M.C., 1995. Hormonal responses to aggression vary in different types of agonistic encounters in male tree lizards, *Urosaurus ornatus*. Horm. Behav. 29, 85–105.

Knapp, R., Moore, M.C., 1996. Male morphs in tree lizards, *Urosaurus ornatus*, have different delayed hormonal responses to aggressive encounters. Anim. Behav. 52, 1045–1055.

Knapp, R., Moore, M.C., 1997. Male morphs in tree lizards have different testosterone responses to elevated levels of corticosterone. Gen. Comp. Endocrinol. 107, 273–279.

Moore, I.T., Wada, H., Perfito, N., Busch, D.S., Hahn, T.P., Wingfield, J.C., 2004a. Territoriality and testosterone in an equatorial population of rufous-collared sparrows, *Zonotrichia capensis*. Anim. Behav. 67, 411–420.

Moore, I.T., Walker, B.G., Wingfield, J.C., 2004b. The effects of combined aromatase inhibitor and anti-androgen on male territorial aggression in a tropical population of rufous-collared sparrows, *Zonotrichia capensis*. Gen. Comp. Endocrinol. 135, 223–229.

Morris, J.A., Jordan, C.L., Breedlove, S.M., 2004. Sexual differentiation of the vertebrate nervous system. Nat. Neurosci. 7 (10), 1034–1039.

Nespor, A.A., Lukazewicz, M.J., Dooling, R.J., Ball, G.Fl., 1996. Testosterone induction of male-like vocalizations in female budgerigars (*Melopsittacus undulates*). Horm. Behav. 30, 162–169.

Nottebohm, F., 1980. Testosterone triggers growth of the vocal control nuclei in adult female canaries. Brain Res. 189, 429–436.

Sapolsky, R., 1989. Hypercortisolism among socially subordinate wild baboons originates at the CNS level. Arch. Gen. Psychiatry 46, 1047–1051.

Schlinger, B.A., 1998. Sexual differentiation of avian brain and behavior: current views on gonadal hormone-dependent and independent mechanisms. Annu. Rev. Physiol. 60, 407–429.

Schlinger, B.A., Lane, N.I., Grisham, W., Thompson, L., 1999. Androgen synthesis in a songbird: a study of Cyp17 (17a-Hydroxylase/C17,20-Lyase) activity in the zebra finch. Gen. Comp. Endocrinol. 113, 46–58.

Schlinger, B.A., Schultz, J.D., Hertel, F., 2001. Neuromuscular and endocrine control of an avian courtship behavior. Horm. Behav. 40, 276–280.

Schultz, J.D., Schlinger, B., 1999. Widespread accumulation of [^{3}H] testosterone in the spinal cord of a wild bird with an elaborate courtship display. Proc. Natl. Acad. Sci. 96, 10428–10432.

Schultz, J.D., Hertel, F., Bauch, M., Schlinger, B.A., 2001. Adaptations for rapid and forceful contraction in wing muscles of the male golden-collared manakin: sex and species comparisons. J. Comp. Physiol., A Sens. Neural Behav. Physiol. 187, 677–684.

Silver, R., Ball, G.F., 1989. Brain, hormone, and behavior interactions in avian reproduction: status and prospectus. Condor 91, 966–978.

Stein, A.C., Uy, J.A., 2006. Plumage brightness predicts male mating success in the lekking golden-collared manakin, *Manacus vitellinus*. Behav. Eco. 17, 41–47.

Walsh, P.S., Metzger, D.A., Higuchi, R., 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10, 506–513.

Summers, C.H., 2002. Social interaction over time, implications for stress responsiveness. Integ. and Comp. Biol. 42, 591–599.

Wikelski, M., Hau, M., Wingfield, J.C., 1999. Social instability increases plasma testosterone in a year-round territorial neotropical bird. Proc. R. Soc. London, Ser. B Biol. Sci. 266, 551–556.

Wikelski, M., Hau, M., Robinson, W.D., Wingfield, J.C., 2003. Reproductive seasonality of seven neotropical passerine species. The 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., Jacobs, J.D., Soma, K., Maney, D.L., Hunt, K., Wisti-Peterson, D., Meddle, S.L., Ramenofsky, M., Sullivan, K., 1999. Testosterone, aggression and communication: ecological bases of endocrine phenomena. In: Konishi, M., Hauser, M. (Eds.), The Biology of Communication. MIT Press, Cambridge, MA. Vol.