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TESTOSTERONE DOES NOT INCREASE IN RESPONSE TO CONSPECIFIC CHALLENGES IN THE WHITE-BELLIED ANTBIRD (*MYRMECIZA LONGIPES*), A RESIDENT TROPICAL PASSERINE

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Abstract.—Resident tropical passerines that exhibit year-round territorial aggression do not fit well into the temperate-zone model, because testosterone does not increase substantially during the breeding season. We studied patterns of testosterone secretion in the White-bellied Antbird (*Myrmeciza longipes*), a resident tropical species in Panama that maintains territories year-round and is capable of aggression throughout the year, regardless of its stage of reproduction. Levels of plasma testosterone were low (mean = 0.30 ng mL^{-1}) throughout the breeding and nonbreeding seasons and did not differ between them. Testosterone also did not increase in response to simulated conspecific intrusions. When we used temporary removal experiments to induce natural, extended conflict between males, testosterone levels did not increase in response to the extended social instability that resulted. White-bellied Antbirds demonstrate an apparent uncoupling of testosterone and territorial aggression throughout the year. *Received 6 September 2004, accepted 11 May 2005.*

Key words: *Myrmeciza longipes*, territoriality, testosterone, tropical birds, White-bellied Antbird.

La Testosterona no Aumenta como Respuesta a Desafíos de Individuos Coespecíficos en *Myrmeciza longipes*, un Paserino Residente de la Zona Tropical

Resumen.—Las aves paserinas residentes de la zona tropical que exhiben agresión territorial a lo largo del año no encajan correctamente en el modelo de la zona templada, pues su testosterona no se incrementa de modo sustancial durante la época reproductiva. Estudiamos los patrones de secreción de testosterona en *Myrmeciza longipes*, una especie tropical residente en Panamá que maintiene territorios a través del año independientemente de su estado reproductivo. Los niveles de testosterona en el plasma fueron bajos (media = 0.30 ng mL^{-1}) y no difirieron entre la época reproductiva y la no reproductiva. La testosterona no aumentó como respuesta a intrusiones territoriales simuladas. Cuando empleamos experimentos de remoción temporal para inducir la ocurrencia natural de conflictos extendidos entre machos, los niveles de testosterona no aumentaron como respuesta a la inestabilidad social extendida que se generó. *Myrmeciza longipes* parece exhibir un desacoplamiento entre la testosterona y la agresión territorial durante todo el año.

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THE STEROID HORMONE testosterone has been shown to have an important influence on the behavior of birds. In most temperate-zone birds, an increase in testosterone, testes size, territoriality, aggression, and reproduction all coincide during a brief period in the spring (for overviews, see Balthazart 1983, Wingfield et al. 1990, Ketterson et al. 1992, Wingfield 1994). Wingfield et al. (1990) proposed the "challenge hypothesis," which predicts that in socially monogamous species, territorial challenges should increase testosterone, which would function to boost an individual's persistence during periods of social instability (challenges). However, that hypothesis is based generally on temperate species. Tropical bird species that maintain year-round territories do not seem to fit well into this temperate-zone model. Data for tropical birds suggest an uncoupling of territoriality, testosterone, aggression, and reproduction (Wikelski et al. 1999a, b, 2003). Wikelski et al. (1999a) have demonstrated that (1) tropical birds with year-round territories can be aggressive at any time of the year, (2) mating and social systems may play a role in the pattern of testosterone secretion, and (3) testosterone can be high despite entirely regressed gonads. These data hint at a system that is much different from the fairly well-understood temperate-zone model.

Although high levels of testosterone are not typical of tropical birds, testosterone can still have an effect on singing and aggressive behavior. Captive Spotted Antbirds (*Hylophalyx naevioides*) with experimentally increased testosterone levels showed higher levels of aggression (Hau et al. 2000). Wikelski et al. (1999b) have also shown that testosterone levels will increase in Spotted Antbirds after extensive (2 h) simulated intrusions using playbacks of conspecific songs. Bay Wrens (*Thryothorus nigricapillus*) are another tropical species that are highly aggressive year-round but do not have measurable levels of testosterone (Levin and Wingfield 1992). Bay Wrens had low levels of testosterone, even when individuals were exposed to simulated territorial intrusions (Levin 1996). The uncoupling of territorial aggression and testosterone is emerging as a common theme in tropical passerines (Wingfield et al. 1991, 1992; Wingfield and Lewis 1993; Wikelski et al. 2003; Moore et al. 2004).

We studied patterns of testosterone secretion in a tropical passerine species in Panama, the White-bellied Antbird (*Myrmeciza longipes*), a resident species that exhibits aggressive territorial behavior year-round. In another component of this research, we found that males are not more aggressive during the breeding season than during the nonbreeding season (Fedy and Stutchbury 2005). Given these results and the findings of previous studies on year-round territorial species, we expected that testosterone levels would not vary between nonbreeding and breeding life-stages. We also predicted that testosterone levels would not correlate with the duration of simulated territorial intrusions using playbacks of conspecific songs. We used removal experiments in an attempt to create extended social disruption in a natural context. We temporarily removed territorial males until a replacement male bird moved into their territory and began duetting with the widowed female. We then released the removed individual back onto his territory, which resulted in the male competing with the replacement individual to regain his original territory. This created a situation of extended challenge for the focal birds. We then captured the males and measured testosterone levels <24 h after the release of the original territory owner to determine whether or not these extended natural challenges resulted in an increase in testosterone levels.

METHODS

Study site.—Our study was conducted during 2000 and 2001 in Parque Nacional de Soberania, Panama (9°7′N, 79°40′W). The area is a tropical lowland forest located at the junction of the Panama Canal and the Chagres River and receives ~2.6 m of rainfall per year (Karr 1971). Most of the precipitation occurs during the wet season (May–December). The remainder of each year is a pronounced dry season (Windsor 1990), during which litter-arthropod populations are significantly lower than in the wet season (Levings and Windsor 1982). The nonbreeding season for ground-foraging insectivorous birds in the area corresponds to the dry season (approximately January to April). Breeding can begin in late April, when the wet season begins and litter arthropod populations increase; however, breeding is highly asynchronous, and evidence of breeding can still be found into December (Robinson et al. 2000).

Study species.—White-bellied Antbirds are a medium-sized (29 g) passerine in the Formicariidae and are found in second-growth and edge habitat from northern Brazil to central Panama (Meyer de Schauensee 1966, Wetmore 1972, Fedy and Stutchbury 2004). White-bellied Antbirds feed on litter arthropods and often maintain territories and associate in pairs throughout the year. We used conservative definitions of the nonbreeding and breeding seasons for White-bellied Antbirds. Samples classified as nonbreeding were collected from February to March each year, and those considered breeding samples were collected from late May to July 2000.

Birds were caught in mist nets by broadcasting a conspecific song on their territory. Individuals were color-banded, and a small (~100 μL) blood sample was taken from the brachial vein. Blood was collected in heparinized capillary tubes, stored on ice, and centrifuged within 5 h. Plasma was collected, frozen, and transported back to the laboratory in Toronto on dry ice. Songs used in the playbacks were high-quality recordings of several territorial males in neighboring study areas, and recordings mimicked the typical song rate of aggressive individuals. Territory boundaries were determined by mapping bird locations while they sang and disputed borders, and by assessing responses to playback of recorded songs (Falls 1981). Several birds were also radiotagged and tracked as part of a different study, and telemetry locations contributed to determination of territory boundaries.

Removals.—Five territorial males were temporarily removed from their territories and kept in captivity for four days to induce social instability. A male was considered replaced during those four days if a different male began singing on the territory and duetting with the female. The original territory owner was released back onto his territory in the afternoon of the fourth day. The original territory owner and replacement bird were then caught the following morning, and a blood sample was taken from each bird to measure plasma testosterone levels. For a more detailed explanation of removal methods, see Fedy and Stutchbury (2004).

Testosterone analysis.—Plasma levels of testosterone were measured using a Coat-A-Count Total Testosterone kit (Diagnostic Products, Los Angeles, California). The procedure is a solid-phase radioimmunoassay in which ^{125}I-labeled testosterone competes with testosterone in the plasma sample for antibody sites. The bound and free testosterone are separated and counted in a gamma counter. The testosterone level is determined from the calibration curve. The antiserum is highly specific for testosterone, with very little cross-reactivity with other androgen hormones. Samples below the detection limit (26 of 35 samples = 74%) were set at 0.2 ng mL^{-1}, representing the highest possible value and following Wikelski et al. (1999a).

All data were analyzed using SPSS, version 10 (SPSS, Chicago, Illinois). Data were presented as means ± SE, and tests were considered significant at $P < 0.05$. Statistical tests were presented on a case-by-case basis.

Results

Seasons.—Plasma testosterone levels in male White-bellied Antbirds did not differ between nonbreeding (0.31 ± 0.06 ng mL^{-1}, $n = 20$) and breeding (0.37 ± 0.08 ng mL^{-1}, $n = 15$) seasons (Mann-Whitney U-test, $Z = -0.890$, $P = 0.37$). There was no significant difference between seasons in the likelihood of obtaining a detectable level of plasma testosterone ($\chi^2 = 0.798$, $P = 0.372$) and, therefore, our ability to detect testosterone would not have affected the above results.

Simulated intrusions.—We tested whether simulated intrusions affect plasma testosterone levels in White-bellied Antbirds. We excluded individuals involved in removal experiments in this analysis to avoid any confounding effects of the resulting social instability. We did not find any relationship between duration of playback and plasma testosterone levels (linear regression: $r^2 = 0.024$, $F = 0.69$, $P = 0.412$, $n = 30$; Fig. 1).

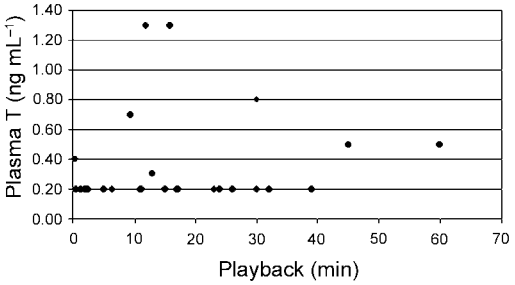

Fig. 1. Plasma testosterone concentrations were not related to the duration of playbacks.

Removals and social instability.—To analyze the potential effect of extreme social instability on plasma testosterone levels, we compared testosterone levels of individuals experiencing social instability as a result of removals in the nonbreeding season (n = 5) with those of individuals captured by playback under otherwise stable social situations in the nonbreeding season (n = 15). No significant difference existed between plasma testosterone levels of those individuals experiencing extreme social instability because of removals and replacements (0.30 ± 0.10, n = 5) and those under stable conditions (0.31 ± 0.08; Mann-Whitney U-test, Z = –0.062, P = 0.95, n = 15).

Discussion

White-bellied Antbirds have very low circulating levels of testosterone under all circumstances. Other tropical, year-round territorial and socially monogamous species have similarly low levels (Wikelski et al. 2003). In White-bellied Antbirds, circulating testosterone does not appear to regulate territorial aggression. We found no variation in testosterone levels between breeding and nonbreeding seasons. Further evidence of the uncoupling of testosterone and aggression is highlighted, in that male White-bellied Antbirds are actually more aggressive during the nonbreeding season (Fedy and Stutchbury 2004).

Levels of circulating testosterone were also not affected by the length of the simulated territorial intrusions. Even relatively long playbacks (1 h) did not increase testosterone levels. Wikelski et al. (1999a) found that testosterone increased only after 2 h of simulated intrusions. It is possible that testosterone levels may have increased in White-bellied Antbirds had we continued our simulated intrusions for >2 h; however, this is unlikely, given that testosterone did not increase even after the extended conflicts that resulted from our removal experiments.

Our study is unique in that we were able to create natural extended conflict between male birds by creating social instability through removal experiments. This type of experiment recreates confrontations as they would occur naturally, allowing a unique opportunity to study the influence of social instability on testosterone levels. Territory switching occurs quite commonly in White-bellied Antbirds (Fedy and Stutchbury 2004), and removal experiments mimicked that type of confrontation. Despite the presence of direct competition with another male, testosterone levels did not increase.

Our study found very low levels of circulating plasma testosterone; however, this does not rule out all possible ways that testosterone may influence aggression. If the sex steroid precursor dehydroepiandrosterone (DHEA) is present in large enough quantities in the circulation, it may be transferred into testosterone in the brain during territorial challenges. In other avian species that exhibit year-round aggression, concentrations of DHEA were correlated with levels of aggression (Soma and Wingfield 2001, Hau et al. 2004). Furthermore, though this is uncommon, White-bellied Antbirds may be able to limit the "leakage" of testosterone into circulation, thus limiting circulating plasma T to very low levels.

White-bellied Antbirds demonstrate an apparent uncoupling of territorial aggression and testosterone, as has been found in similar species. It seems that the "challenge hypothesis" may not be the best explanation for the patterns of testosterone secretion observed in tropical year-round territorial birds (Goymann et al. 2004). The mating systems of temperate passerine species may explain the elevated levels of testosterone in relation to tropical species, influenced in particular by the presence of extrapair copulations (Stutchbury and Morton 2001, Gill et al. 2005). Currently, this hypothesis is difficult to test, given the limited data on both patterns of testosterone secretion and rates of extrapair copulations in tropical year-round passerines. More studies in this area may reveal that a species' mating system may have the greatest influence on patterns of testosterone secretion in passerines.

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