

Territorial aggression and hormones during the non-breeding season in a tropical bird

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Abstract

The hormonal control of territorial aggression in male and female vertebrates outside the breeding season is still unresolved. Most vertebrates have regressed gonads when not breeding and do not secrete high levels of sex steroids. However, recent studies implicate estrogens in the regulation of non-breeding territoriality in some bird species. One possible source of steroids during the non-breeding season could be the adrenal glands that are known to produce sex steroid precursors such as dehydroepiandrosterone (DHEA). We studied tropical, year-round territorial spotted antbirds (*Hylophylax n. naevioides*) and asked (1) whether both males and females are aggressive in the non-breeding season and (2) whether DHEA is detectable in the plasma at that time. We conducted simulated territorial intrusions (STIs) with live decoys to male and female free-living spotted antbirds in central Panama. Non-breeding males and females displayed robust aggressive responses to STIs, and responded more intensely to decoys of their own sex. In both sexes, plasma DHEA concentrations were detectable and higher than levels of testosterone (T) and 17 β -estradiol (E₂). In males, plasma DHEA concentrations were positively correlated with STI duration. Next, we conducted STIs in captive non-breeding birds. Captive males and females displayed robust aggressive behavior. Plasma DHEA concentrations were detectable in both sexes, whereas T was non-detectable (E₂ was not measured). Plasma DHEA concentrations of males were positively correlated with aggressive vocalizations and appeared to increase with longer STI durations. We conclude that male and female spotted antbirds can produce DHEA during the non-breeding season and DHEA may serve as a precursor of sex steroids for the regulation of year-round territorial behavior in both sexes.

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Introduction

Numerous studies have demonstrated that the steroid hormone testosterone (T) regulates territorial aggression during the breeding period. In birds and other vertebrate taxa, plasma titers of T are positively correlated with the expression of aggressive behavior in socially unstable contexts, and experimental manipulations of circulating T levels change aggressiveness (e.g., Balthazart, 1983; Harding, 1981; Nelson, 2000; Wingfield et al., 1990). In contrast, bird species that exhibit year-round, non-breeding (winter) or female territorial aggression do not show a consistent

association of plasma T levels and aggression (summaries in Dittami and Gwinner, 1990; Levin and Wingfield, 1992; Schwabl, 1992; Soma and Wingfield, 1999; Wikelski et al., 1999b; Wingfield, 1994b; Wingfield et al., 2001). Moreover, castration does not diminish aggressive behavior in some winter-territorial birds (Pinxten et al., 2000; Wingfield, 1994a).

Thus, the same behavior, territorial aggression, expressed at different times in the life cycle may have different control mechanisms (Wingfield and Soma, 2002; Wingfield et al., 2001). Elevated plasma T levels may incur physiological ‘costs’ and thus birds may use alternative mechanisms for the hormonal regulation of aggression outside the breeding season (e.g., Hillgarth and Wingfield, 1997; Ketterson et al., 1996; Wingfield et al., 2001). However, new studies in which sex steroid synthesis or receptor binding was pharmacologically inhibited did reduce non-breeding territorial

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aggression in some bird species (Soma et al., 1999, 2000a,b; but see Canoine and Gwinner, 2002). This suggests that T may still be involved in the regulation of non-breeding territorial aggression, but in an indirect manner. T can affect behavior either directly by binding to androgen receptors or indirectly by conversion into 17β -estradiol (E_2) by the enzyme aromatase (e.g., Schlinger and Callard, 1990). Indeed, experiments involving the blockade of E_2 synthesis and the subsequent replacement of this hormone in song sparrows (*Melospiza melodia morphna*) suggest that it is E_2 that plays a role in male aggression during the non-breeding season (Soma et al., 2000a,b). During the breeding season, the gonads are the primary producers of sex steroids, but in most species, gonads regress during the non-breeding season (Murton and Westwood, 1977; Wingfield and Farner, 1993). If sex steroids are involved in regulating aggression during the non-breeding season, where do they originate?

A growing number of observations suggest that non-gonadal tissue can contribute to circulating steroid hormone levels (Adkins-Regan et al., 1990; Boswell et al., 1995; Marler et al., 1988; Schlinger et al., 1999; Wikelski et al., 1999c). One precursor of sex steroids, dehydroepiandrosterone (DHEA), is beginning to gain attention in this context (Soma and Wingfield, 1999). DHEA may be produced by the adrenals in some avian species (Freking et al., 2000; Schlinger et al., 1999; Soma et al., 2002a), and thus could provide a substrate for sex steroid-producing enzymes in the brain year-round (Soma and Wingfield, 1999). Indeed, DHEA has been detected in the plasma of song sparrows and quail (*Coturnix japonica*; Soma and Wingfield, 2001; Tsutsui and Yamazaki, 1995). Furthermore, in song sparrows, the administration of exogenous DHEA during the non-breeding season stimulates song during aggressive encounters (Soma et al., 2002a).

Here, we examine whether sex steroid precursors are involved in the endocrine control of year-round territorial aggression (Hau, 2001; Hau et al., 2000; Soma and Wingfield, 1999) in males and females of the spotted antbird (*Hylophylax n. naevioides*) from central Panama. Spotted antbirds are truly year-round territorial, occupying the same multi-purpose territory over many years (Willis, 1972). Males and females establish long-term pair bonds and both sexes actively defend the territory with song and aggressive displays (Bard et al., 2002; Hau et al., 2000; Wikelski et al., 1999a, 2000; Willis, 1972).

Despite being territorial year-round, spotted antbirds usually have low to non-detectable plasma T concentrations (around 0.3 ng/ml), even during the breeding season (Hau et al., 2000; Wikelski et al., 1999a, 2000). Nevertheless, experimental studies indicated a role for T, or its estrogenic metabolites, in the regulation of male territoriality. First, during periods of social instability, that is, when territorial intrusions by conspecifics occur, spotted antbird males have increased plasma T titers (Wikelski et al., 1999a). Such increases can also be found during the non-breeding season when gonads are regressed, and typically occur when terri-

torial challenges include multiple males or exceed a duration of 140 min (Wikelski et al., 1999a). Second, administration of exogenous T (during the non-breeding season) enhances aggressive behavior (Hau et al., 2000). Third, a combined treatment with an androgen receptor antagonist and an aromatase inhibitor during the breeding season diminishes aggressive behavior (Hau et al., 2000). Female spotted antbirds are also territorial and sing year-round (Wikelski et al., 2000; Willis, 1972), but so far no increases in plasma T levels have been found (Wikelski et al., 2000).

We conducted two experiments during the non-breeding season, when spotted antbirds have fully regressed gonads (Wikelski et al., 2000). We first did a field study in which we quantified levels of aggressiveness and circulating concentrations of steroid hormones (DHEA, T and E_2) and luteinizing hormone (LH) in both sexes after simulated territorial intrusions (STIs) of varying durations. Second, we brought males and females into captivity to investigate under controlled conditions whether prolonged aggressive encounters affect plasma steroid concentrations. We exposed captive birds to two kinds of STIs: (1) multi-male STIs involving five birds simultaneously, to simulate the situation at an army ant swarm where interactions between territory owners are particularly intense (see below), and (2) dyadic STIs as in the field experiment, involving only two males. Captive STIs were conducted for either 30 or 160 min, aggressive behavior was quantified and circulating steroid levels were determined after the STI.

Materials and methods

Subjects

Spotted antbirds are small suboscine passerines (Family *Thamnophilidae*, Ridgely and Tudor, 1994). In Soberania National Park, Republic of Panama (9°N), these birds breed during the rainy season from about May to October each year (Hau et al., 1999; Wikelski et al., 2000; Willis, 1972). Spotted antbirds are pure insectivores and facultatively follow army ant swarms while foraging, during which they frequently intrude onto neighboring territories (Wikelski et al., 1999a; Willis, 1972).

All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (IACUC protocol no. 99–031, for Experiment 1) and Princeton University IACUC (protocol no. 1430, for Experiment 2) and met all applicable state and federal guidelines.

Experiment 1: Simulated territorial intrusions to free-living birds

We conducted STIs (Wingfield, 1985) to free-living non-breeding birds during January 11–30, 1999. As expected, spotted antbirds did not nest at this time (C. Edwards, personal communication). We worked on known territories

in Soberania National Park in fairly mature forest areas, where visibility in the understory was at least 20 m.

We conducted STIs using caged live decoys while simultaneously broadcasting playback of recorded song. One male and one female spotted antbird were each used as decoy. A live song wren (*Cyphorhinus phaeocephalus*) served as a decoy for control trials. Spotted antbirds may forage in association with song wrens, and thus are familiar with these birds in a non-territorial context. For song playback, we used tape-recorded song from three randomly selected individuals for each species and sex. Each tape consisted of the repeated song recordings of one individual bird, repeated every 12 s for spotted antbirds (song duration, 4–5 s) and every 4 s for song wrens (song duration about 2 s), reflecting natural song patterns (Bard et al., 2002).

Before the start of the trials, two to four mistnets were set up but remained closed. The cage and speakers were placed in the approximate center of the territory, the cage cover was removed and the playback started. If territory owners did not respond within 15 min of starting the playback, the STI was stopped on the assumption that birds were out of hearing distance and a STI was repeated on this location on another day. After 30 min, the STI was terminated and the decoy was removed. Nets were opened and territory owners were caught using conspecific playback, using a different tape than during the preceding STI. We also caught one female and six males that were not included in STI or control trials, using conspecific playback only (with no decoy present). Spotted antbirds express robust aggressive behavior when exposed to only playback (without a decoy, Wikelski et al., 1999a). We therefore express playback durations as the sum of the STI (30 min) plus the time a bird was exposed to conspecific playback (without decoy) until it was caught.

Most trials involved previously color-banded birds. Unbanded birds (e.g., new territory owners) received a unique combination of a numbered aluminum ring and three-colored leg bands. A small blood sample was taken immediately after capture (within 5–10 min, see below). We measured tarsus length, body mass, cloacal protuberance length, furcular fat stores, molt and brood patch. A random subset of birds was laparotomized under Isoflurane anesthesia as described previously (Hau et al., 1998, 2000) to confirm that gonads were regressed. Birds were then released back onto their territory.

Experiment 2: Simulated territorial intrusions to captive birds

Between 19 and 24 Dec 2000, we caught 21 male and 4 female non-breeding spotted antbirds and one song wren in Soberania National Park. Birds were placed in individual cages that were visually isolated from each other. Cages were kept in an indoor room at the Gamboa field station of the Smithsonian Tropical Research Institute (STRI), under a photoperiod simulating natural condition (for general bird maintenance, see Hau et al., 2000). STIs were carried out

between Dec 27, 2000 and Jan 9, 2001, from 7:30 a.m. to 5 p.m. One male died of unknown causes before completion of all STIs and therefore was removed from all analyses. On Jan 21, 2001, all birds were laparotomized.

Captive STIs were carried out either in a noise-proof chamber or in a basement room about 200 m away from the general bird holding area. All multi-male STIs were conducted in the soundproof chamber; dyadic STIs were conducted in one of the rooms at random. For a ‘multi-male STI,’ the cages of four males were placed opposite each other. One female was always included in multi-male trials because previous experiments have shown that the presence of a female enhances aggressive behavior (Hau et al., unpublished data). Following the completion of multi-male STIs, we conducted ‘dyadic’ STIs, for which the cages of only two males were placed opposite of each other.

For multi-male STIs, two walkman connected to two active speakers each played conspecific song from two different individuals, for the dyadic STI only one playback setup was used. Two video cameras were set up such that they recorded the birds’s behavior for the first 10 s of each minute (in the multi-male trials, one camera recorded the behavior of two birds simultaneously). Cages were brought into the room with the fronts covered and birds were allowed to accommodate to the situation for at least 10 min. Next, front covers were removed, playback and video recording started and the experimenter left the room.

Immediately after termination of the STIs, all birds had a blood sample taken (within less than 30 min in the multi-male trials, within 5–10 min during the dyadic STIs), were weighed and had the condition of their breast muscle and their furcular fat reserves scored. Birds were then placed back into their cages and returned to the holding room.

All males were subject first to a multi-male STI for either 30 min ($n = 4$) or 160 min ($n = 16$). They were then subject to dyadic STIs. For these, males were divided into two groups ($n = 10$ each): each bird was tested both in a song wren and a conspecific STI (in random order), but one group of birds was exposed only to 30 min dyadic STIs and the second group of birds only to 160 min dyadic STIs. Hence, each male was tested three times: first, during a multi-male STI (Dec 27–29, 2000), and then during a hetero- and a conspecific dyadic STI (during Jan 2–4 and Jan 7–9, 2001, respectively). Repeated tests of one individual were separated by at least 5 days. Birds were assigned to treatments and groups at random, ensuring that during each STI birds were naïve to each other.

On Jan 5, 2001, one multi-female STI was conducted using four females as opponents and playing back the recordings of two different female individuals. Females were not included in any dyadic STIs.

Aggressive behaviors

We classified spotted antbird aggressive behaviors according to Willis (1972) and our own observations (Bard

et al., 2002; Hau et al., 2000). Sonograms of songs and calls are published elsewhere (Bard et al., 2002; Willis, 1972). ‘Snarls’ are hissing calls that are only used during highly aggressive displays. They are directed at a close opponent and often occur with a display of the white chest feathers (especially in males) and the white back patch. The white back patch is only exposed during aggressive displays. During aggressive encounters, spotted antbirds are also very active and switch perches frequently. In some trials, birds flew directly at the decoy cage and physically touched it, which was scored as an attack.

In free-living birds, we recorded: latency to show any response (any vocal response, or physical appearance within 20 m of the decoy); latency and numbers of songs, snarls, and attacks on the cage; latency and duration of display of the white back patch; number of flights within 20 m of the decoy; closest approach to the cage; time spent within 5 or 10 m of the cage; and time not visible.

For captive STIs, we analyzed each 10-s segment for 30 min STIs, and the first and last 30 min of each 160 min STI from the videotapes. We counted number of songs, snarls, chips, hops, and whether the white back patch was exposed. An ‘attack’ was scored when a bird flew against the front side of its cage. This happened only rarely, was preceded by strong aggressive display from the attacker and was responded by the recipient by hopping to the bottom or to the back of its cage (apparently trying to escape). Behavior was then expressed as $n/30$ min (averaging the first and last 30 min for each individual in 160 min STIs).

Hormone analysis

A small blood sample (150–250 μ l) was taken by puncturing the wing vein with a 26-gauge needle and collecting the blood in heparinized microcapillary tubes (Hau et al., 1998; Wikelski et al., 2000; Wingfield and Farner, 1976). Samples were kept at 4°C until centrifugation (within 4 h). Plasma was aspirated off and frozen at –20°C. Samples were transported to the USA on dry ice under permission of Panamanian and US authorities. All samples from Experiment 1 were analyzed in one radioimmunoassay (RIA); samples from Experiment 2 were analyzed in two separate RIAs. Antibodies were purchased from Endocrine Sciences, Tarzana, CA (DHEA), Wien Laboratories Inc., Flanders, NJ (T) and Biogenesis, Poole, England (E₂). Steroids were partially purified using column chromatography to separate DHEA, T and E₂ according to Soma and Wingfield (2001), with slight modifications as follows. Plasma samples were extracted twice with 3 ml redistilled dichloromethane and dried under a N₂ stream. Resuspended samples were added to diatomaceous earth columns mixed with pure propylene glycol. Then, 2.5 ml of 10% ethyl acetate in iso-octane was added. This fraction was discarded. The DHEA fraction was collected after adding 2.5 ml of 20% ethyl acetate in iso-octane, the T fraction was eluted in 2.0 ml of 40% ethyl acetate in iso-octane, and the

E₂ fraction was collected after adding 2.5 ml of 50% ethyl acetate in iso-octane. Overall recoveries for all three assays were (mean \pm SE) 82.4 \pm 2.7% for DHEA, 64.1 \pm 4.7% for T and 67.7 \pm 1.4% for E₂ (E₂ was only measured for samples from Experiment 1). Lower detection limits were at 0.07–0.1 ng/ml for DHEA, 0.06–0.08 ng/ml for T and 0.12–0.22 ng/ml for E₂. Intra-assay variation was determined by including two sets of cold standards in three different concentrations in each assay. For DHEA, the mean intra-assay coefficient of variation (CV) for the low (0.125 ng/ml), the intermediate (0.25 ng/ml) and the high (0.375 ng/ml) standards were 8.7%, 20.2% and 11.6%, respectively. For T, the CVs (same concentrations) were 16.7%, 8.1% and 3.9%. For E₂, the CVs (same concentrations) were 11.1%, 6.1% and 1.2%. Inter-assay coefficient of variation for the two assays run on the samples from the second experiment were: DHEA (the three cold standards in order of decreasing concentration) 4.9%, 4.0% and 10.9%, respectively, and for T 20.9%, 7.9% and 33.5%, respectively.

LH concentration in samples was determined in a single RIA, using the postprecipitation, double antibody method for avian LH developed by Follett et al. (1972) and Sharp et al. (1987), with minor modifications by T.J. Van’t Hof (Gwinner et al., 2002). The lower detection limit of the assay was at 0.14 ng/ml. The intra-assay CV as determined from nine replicates of a chicken plasma pool (at 2 ng/ml) was 6.1%.

Data analysis

Data were analyzed using SPSS 10.0 for Windows (SPSS Inc., Chicago) and presented as means \pm standard errors (SEM). Hormone samples below the detection limit of our assays were set at detection limit as a conservative estimate for statistical analysis. General linear models (GLM) were used whenever possible and some data sets were natural log (ln) transformed to meet the assumptions for use of GLM. All post-hoc tests were Dunnett’s T3. Many of the behavioral data were analyzed using non-parametric tests (as indicated in the text). Only two-tailed tests were used.

Results

Experiment 1: STIs to free-living birds

Aggressive behavior

Aggressive responses of spotted antbirds to STIs were highly species-specific: no bird showed any aggressive behavior during the 10 control trials involving a song wren as a decoy, while robust territorial aggression was displayed by a total of 17 male and 11 female territory owners during 18 conspecific STIs. Spotted antbirds are monogamous and paired year-round (Willis, 1972). Assuming that all birds were paired, the response rate of male territory owners to STIs of any sex was 97% and that of females 61%. In 55%

of all STIs both partners of a pair responded, in 38% only the male responded and in 5% only the female responded.

Male and female territory owners showed similar responses to STIs with decoys of either sex in their latency to show any kind of response (Fig. 1b; ANOVA: effects of sex of decoy or sex of territory owner, both $P > 0.15$, but a trend for a significant interaction: $F_{(1,24)} = 3.4$, $P = 0.077$) and in their song frequency (ANOVA, all tests $P > 0.2$). Behavioral responses of male and female territory owners to STIs with decoys of either sex were also similar for number of flights, closest approach, time spent in 5 m and time not visible.

However, birds did distinguish between ‘intruders’ of different sexes when displaying specifically aggressive behaviors. Of the eight STIs with a male and 10 STIs with a female decoy that were completed, STIs with a male decoy always elicited a response from male territory owners (8/8), compared to only 37.5% (3/8) of female territory owners (Fisher’s exact test, $P < 0.026$, Fig. 1a). Interestingly, both sexes responded equally often to STIs with a female decoy (8/10 males vs. 9/10 females; Fisher’s exact test, $P = 1.0$, Fig. 1a). Males snarled and showed their white back patch significantly faster when presented with a male vs. a female decoy (ANOVA, snarls: effect of sex of decoy: $F_{(1,24)} = 5.8$, $P = 0.024$; white back patch: effect of sex of decoy: $F_{(1,20)} = 8.5$, $P = 0.009$). Males also snarled more and had their back patch exposed more often during STIs with male decoys than with female decoys (ANCOVA, snarls: effect of sex of decoy: $F_{(1,23)} = 11.9$, $P = 0.002$; white back patch: effect of sex of decoy: $F_{(1,20)} = 18.7$, $P < 0.005$; Fig. 1c). Females never snarled, attacked or showed their white patch during male STIs, but did exhibit these behaviors during female STIs (Figs. 1c, d) with an intensity undistinguishable from that of males. Both sexes spent more time in 10 m during STIs with decoys of their own sex, respectively (ANOVA: interaction of sex of territory owner and sex of decoy: $F_{(1,27)} = 5.3$, $P = 0.03$). There were no differences in the attack rate on the decoy cages of male and female territory owners during STIs with different sexes (all tests $P > 0.4$), but attacks were also generally rare.

Hormonal responses

We captured a total of 15 males and 4 females, after control trials, conspecific STIs or random capture using conspecific playback. As expected, spotted antbirds had regressed gonads (testis volumes $1.01 \pm 0.21 \text{ mm}^3$, $n = 4$; follicle diameters $0.3 \pm 0.1 \text{ mm}$, $n = 3$) and small cloacal protuberances (males: $1.64 \pm 0.13 \text{ mm}$, females: $1.8 \pm 0.2 \text{ mm}$; similar to non-breeding birds in Wikelski et al., 2000). No bird had an active brood patch or was molting.

Male and female hormone levels did not differ (ANOVAs for DHEA and LH, Mann–Whitney tests for T and E_2 , all $P > 0.2$, Fig. 2). Plasma LH concentrations in both sexes were low (males: $0.75 \pm 0.12 \text{ ng/ml}$; females: $0.84 \pm 0.23 \text{ ng/ml}$). Plasma DHEA concentrations were detectable and elevated in all males, but their plasma T and E_2 titers were

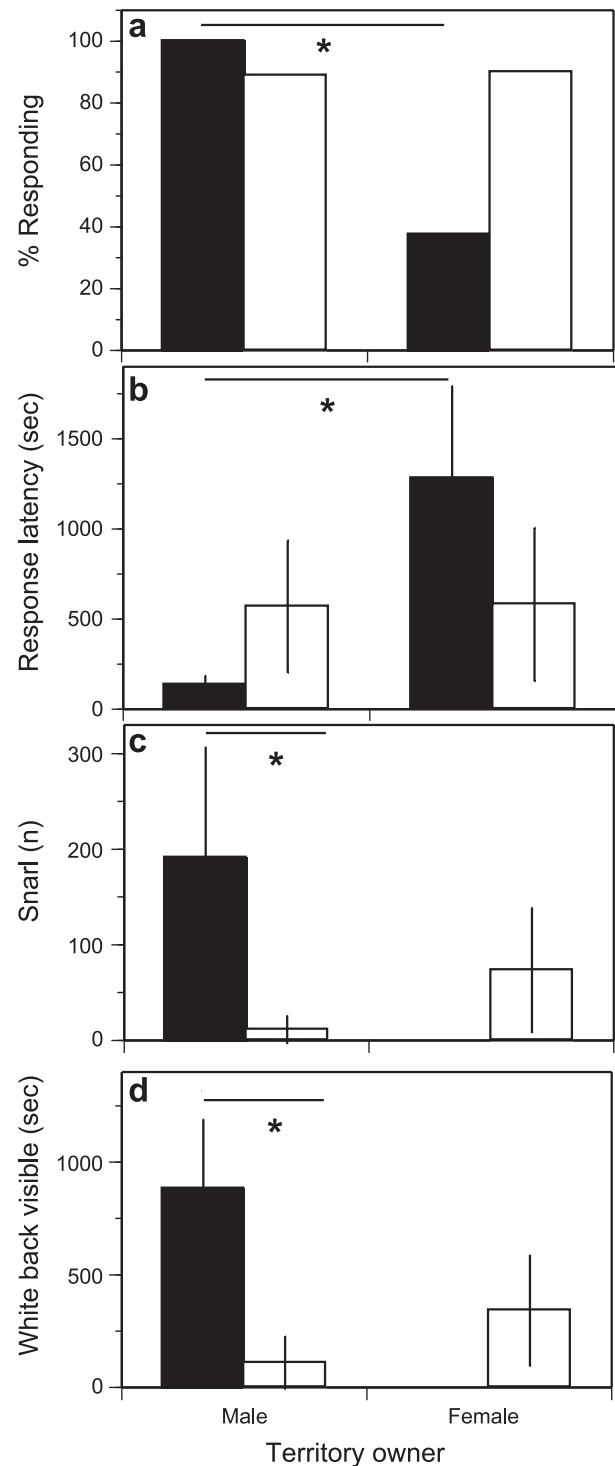


Fig. 1. Aggressive responses (mean \pm SEM) of male and female free-living spotted antbirds to simulated territorial intrusions (STI) for 30 min during the non-breeding season using either a male (black bars, $n = 8$) or a female (white bars, $n = 10$) decoy. (a) Percentage of male and female territory owners responding, (b) latency to show any response, (c) number of snarls, and (d) time in which their white back patch was exposed. Responses of male territory owners appear on the left side of both graphs, while female responses are shown on the right side. Asterisks indicate significant differences, $P < 0.05$.

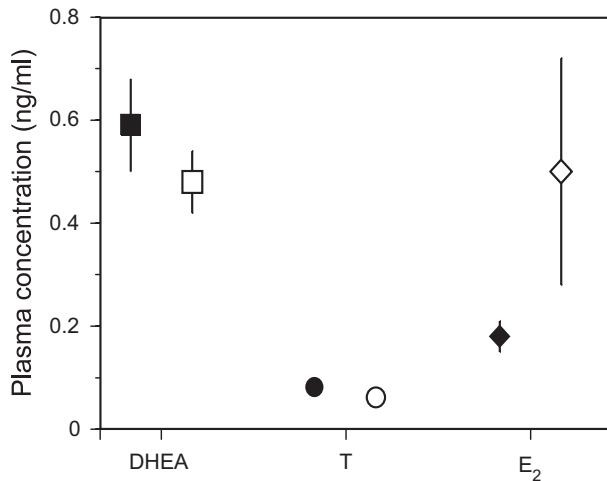


Fig. 2. Plasma concentrations of DHEA, T, and E₂ of male (filled symbols, $n = 15$) and female (open symbols, $n = 4$) free-living spotted antbirds (mean \pm SEM). Error bars for T are small and not visible.

low and mostly undetectable (Fig. 2; only 26.6% samples detectable for T, and 53% for E₂, respectively). All female plasma DHEA concentrations were detectable and in the same range as in males (Fig. 2). Plasma T concentrations of females were all undetectable. Plasma E₂ concentrations were undetectable in one female, low in a second, but elevated in two females with long playback times (0.83 ng/ml after 68 min, and 0.92 ng/ml after 120 min, respectively). Interestingly, the two females with the high E₂ concentrations responded together with their mate, and one of these males also had high E₂ concentrations (0.58 ng/ml).

We could not detect significant differences in plasma hormone concentrations between males caught after different STIs (control vs. conspecific, using male vs. female decoys), or after random playback only. Circulating DHEA concentrations of all males combined were positively correlated with the duration of conspecific playback (total duration including preceding conspecific STI until capture; Pearson's $\rho = 0.65$, $p = 0.008$, Fig. 3a). However, this relationship largely depends on one sample taken after 140 min playback (without the 140 min sample: Pearson's $\rho = 0.295$, $P > 0.3$). There were no significant correlations of playback duration with plasma T, E₂ or LH concentrations (all $P > 0.6$). We were unable to catch many of the birds on which we did STIs with behavioral recordings and therefore cannot test whether correlations exist between behavior and hormones.

Experiment 2: STIs to captive birds

Behavioral and hormonal responses to multi-male STIs

As in previous STI experiments on breeding and non-breeding captive spotted antbirds (Hau et al., 2000), males showed robust aggressive behavior. Males did not change their intensity of behavior between the first and last 30 min of each 160 min encounter (Mann–Whitney tests, all $P >$

0.1), except that the number of hops increased in the last 30 min segment (Mann–Whitney test, $Z = -2.02$, $p = 0.043$). The average intensity of aggressive behavior per 30 min was similar in the 30 min and the 160 min multi-male trials (comparison of number of behaviors/30 min between the two STI durations, Mann–Whitney tests, all $P > 0.1$). On average, males (both STI durations combined) sang 0.29 ± 0.18 times, snarled 20.1 ± 4.78 times, hopped 94.8 ± 14.19 times, showed their back patch 10.49 ± 2.07 times and attacked 0.18 ± 0.09 times.

Male plasma DHEA concentrations were elevated relative to the other sex steroids and were detectable in 19 of 20 individuals (95% detectability). DHEA concentrations tended to be higher after 30 min compared to 160 min multi-male STIs (1.08 ± 0.18 ng/ml vs. 0.79 ± 0.06 ng/ml, respectively, ANOVA, $F_{(1,18)} = 3.53$, $P = 0.076$). Plasma DHEA concentrations of males from both multi-male STI durations combined were significantly correlated with number of snarls/30 min (Pearson's $\rho = 0.522$, $P = 0.018$, Fig.

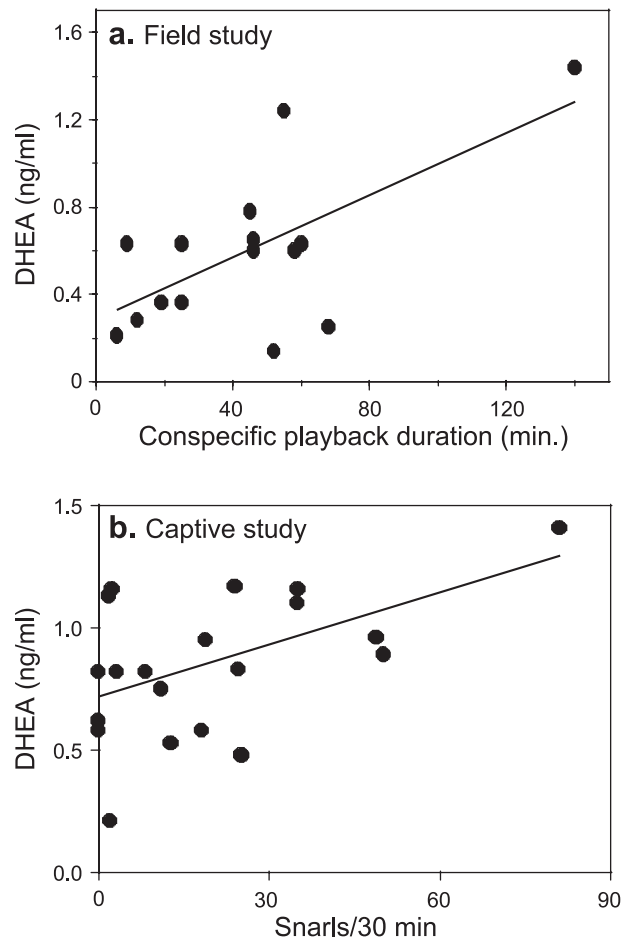


Fig. 3. (a) Correlation of plasma DHEA concentrations of free-living male spotted antbirds with duration of STI and conspecific playback (Pearson's $\rho = 0.65$, $P = 0.008$, $n = 15$). Playback durations are expressed as the sum of the STI (30 min) plus the time the bird was exposed to conspecific playback (without a decoy) until it was caught. (b) Correlation of plasma DHEA concentrations with number of snarls/30 min of captive male spotted antbirds during multi-male STIs (Pearson's $\rho = 0.52$, $P = 0.018$; $n = 20$).

3b). None of the other aggressive behaviors correlated with DHEA. T was non-detectable in all birds (E_2 was not analyzed in the captive birds).

One female was included in each multi-male STI, and one multi-female STI of a duration of 160 min was carried out, which included four females. Females never sang or attacked during these trials, but displayed all other behaviors. As in the field, plasma DHEA concentrations of captive females were similar to those of males. Female T concentrations were all non-detectable.

Behavioral and hormonal responses to dyadic STIs

The behavior of males did not change between the first 30 min and the last 30 min segment of each 160 min encounter (Mann–Whitney tests, all $P > 0.1$), except that hopping activity increased in the last 30 min segment (Mann–Whitney test, $Z = -2.8$, $p = 0.005$). As in field STIs, aggressive behavior in captivity was highly species-specific: birds tended to sing more (conspecific: 0.36 ± 0.19 ; heterospecific: 0.019 ± 0.018 songs per 30 min, Mann–Whitney test, $Z = -1.78$, $p = 0.074$) and snarled significantly more (30.8 ± 9.3 vs. 0.15 ± 0.14 times, $Z = -4.69$, $P < 0.0005$), chipped significantly more (24.23 ± 10.5 vs. 0.38 ± 0.14 times, $Z = -4.06$, $P < 0.0005$) and showed their white back patch more (6.47 ± 1.4 vs. 0.06 ± 0.06 times, $Z = -4.48$, $P < 0.0005$) during conspecific than heterospecific dyadic STIs. Birds tended to hop less during conspecific than heterospecific STIs (40.57 ± 14 vs. 53.19 ± 9.76 times, respectively, Mann–Whitney test, $Z = -1.8$, $P = 0.068$). During conspecific STIs, aggressive behavior was similar in 30 and 160 min dyadic trials (Mann–Whitney tests, all $P > 0.1$), but birds hopped more during 30 min than 160 trials ($Z = -2.1$, $P = 0.034$).

Plasma DHEA concentrations were always detectable and elevated relative to the other sex steroids in captive males. T was again low and only detectable in 3/20 males (16% detectability). Plasma DHEA concentrations of males did not differ between hetero- and conspecific trials (heterospecific STIs: 30 min: 0.8 ± 0.14 ng/ml, 160 min: 0.44 ± 0.77 ng/ml; conspecific STIs: 30 min: 0.58 ± 0.11 ng/ml, 160 min 0.64 ± 0.11 ng/ml; repeated measures ANOVA: hetero- vs. conspecific STI, $P > 0.6$, STI duration $P > 0.5$, but a trend for an interaction between STI type and duration, $F_{(1,17)} = 4.04$, $P = 0.06$). However, when we subtracted plasma DHEA concentrations of each individual after the heterospecific STI from its concentrations after the conspecific STI, males had increased plasma DHEA levels after long compared to short STIs (30 min STIs: -0.22 ± 0.13 ng/ml, 160 min STIs: 0.2 ± 0.14 ng/ml; one-way ANOVA: $F_{(1,17)} = 4.64$, $P = 0.046$).

Discussion

Male and female spotted antbirds responded vigorously to simulated territorial intrusions (STIs) during the non-

breeding season, both in the field and in captivity. Both sexes displayed a similar repertoire of aggressive behaviors, and aggressive behavior was expressed with higher intensity during encounters with an opponent of the same sex. In both experiments, birds had regressed gonads and low plasma concentrations of T and E_2 . Plasma concentrations of DHEA were elevated in males and females, relative to T and E_2 levels. Male plasma concentrations of DHEA were plastic: they varied with playback duration and were positively correlated with aggressive vocalizations.

Aggressive responses to territorial challenges

Free-living male and female spotted antbirds displayed robust and qualitatively similar aggressive behavior towards conspecific intruders during the non-breeding season. However, intruders of the same sex elicited in both males and females a quantitatively stronger response, a higher intensity of aggressive displays and the expression of highly aggressive behaviors such as snarls and attacks (see Fig. 1). A more intense aggressive response to same-sex intruders has been reported for other avian species that defend territories as pairs (e.g., stonechat, *Saxicola torquata*; Gwinner et al., 1994; bay wren, *Thryothorus nigricapillus*, Levin, 1996; dusky antbird, *Cercomacra tyrannina*, Morton and Derrickson, 1996; northern cardinal, *Cardinalis cardinalis*, Yamaguchi, 1998). Higher intra-sexual aggression may occur in monogamous species because same-sex intruders could compete for the long-term mate.

Non-breeding males and females also responded vigorously to STIs in captivity, underscoring the usefulness of this species for studying the regulation of year-round aggressive behavior in both males and females (Hau, 2001). However, in captivity, song activity was reduced, emphasizing the importance of field studies for understanding behavior–hormone relationships.

Hormonal control of aggressive behavior during the non-breeding season

As in previous studies, both free-living and captive spotted antbirds had low plasma concentrations of reproductive hormones (LH, T and E_2 , see Fig. 2; Hau et al., 2000; Wikelski et al., 1999a, 2000). 5α -Dihydrotestosterone (DHT) and androstenedione (AE) are also present in the plasma of spotted antbirds, but are always lower than and correlated with plasma concentrations of T (Wikelski et al., 2000). From our previous work on free-living spotted antbirds, we had expected that territorial intrusions of long durations (>140 min) would increase plasma T concentrations in males (Wikelski et al., 1999a). In contrast, circulating T concentrations remained low in both free-living and captive birds. We cannot fully explain these divergent results, but one possible explanation is that a stronger stimulation than the STIs that we provided is required to increase plasma T concentrations.

However, both sexes had elevated circulating concentrations of DHEA, with average levels 2–5 times higher than those of T and E₂ (Fig. 2). These results demonstrate that male and female spotted antbirds can produce DHEA during the non-breeding season, perhaps in the adrenals or the regressed gonads (both organs contain high concentrations of DHEA in song sparrows during the winter; Soma and Wingfield, 2001). Elevated DHEA concentrations and the lack of a sex difference in plasma DHEA concentrations in spotted antbirds match the year-round expression of aggressive behavior and song in both sexes. In contrast, female song sparrows in winter have lower circulating DHEA concentrations and express reduced aggressive behavior compared to males (Soma and Wingfield, 2001).

Plasma DHEA concentrations of male spotted antbirds appeared to be plastic and positively correlated with several measures of aggressive behavior. Plasma DHEA was positively correlated with playback duration in the field study (Fig. 3a) and with the intensity of aggressive vocalizations in captive multi-male encounters (Fig. 3b). Plasma DHEA concentrations also increased with longer dyadic encounters in captivity, when accounting for levels during hetero-specific STIs. However, there was also a tendency ($p = 0.076$) for DHEA to decrease after long compared to short STIs in the multi-male encounter with captive birds. Similarly, in song sparrows, there was a trend for STIs in autumn to decrease plasma DHEA (Soma and Wingfield, 2001). It is important to keep in mind that circulating hormone concentrations and behaviors are often not correlated in a direct manner (Balthazart, 1983; Nelson, 2000) and caution might be warranted in interpreting the present correlations. Nevertheless, we suggest that our overall findings are consistent with the hypothesis that DHEA regulates aggressive and vocal behavior in non-breeding spotted antbirds. However, further experimental studies are needed to test the causality of this relationship.

There exists evidence from the few studies that have been conducted on other vertebrate species that DHEA is involved in the control of aggressive behavior. In song sparrows, seasonal changes in plasma DHEA match seasonal changes in aggression. Circulating DHEA concentrations are reduced during molt, the life history stage during which males show reduced aggressive behavior (Soma and Wingfield, 2001). In addition, administration of exogenous DHEA via subcutaneous implants increased territorial song in non-breeding male song sparrows (but not other measures of aggression; Soma et al., 2002a). Furthermore, in humans, there is a positive correlation between plasma DHEA concentrations and aggression in young males (e.g., Dmitrieva et al., 2001; van Goozen et al., 1998, 2000).

The current study is, to our knowledge, the first examination of plasma DHEA concentrations in a tropical bird. Previous studies in several species of tropical birds have found non-detectable plasma T throughout the year (Astheimer and Buttemer, 1999; Levin and Wingfield, 1992; Wikelski et al., 2000, 2003; Wingfield et al., 1992),

so the present results on DHEA might be of broad relevance to a variety of tropical birds. The finding of elevated plasma DHEA concentrations support earlier suggestions that this steroid might function as a precursor for active steroids such as T and E₂ in both sexes. In zebra finches and song sparrows, the brain can convert DHEA to T and E₂ (Soma et al., 2002b; Vanson et al., 1996; see also Matsunaga et al., 2001).

Conclusions

A new view is emerging that the regulation of non-breeding, year-round and female territorial aggression may involve ‘non-traditional’ hormonal mechanisms (Hau, 2001; Soma and Wingfield, 1999; Wingfield et al., 1999, 2001). The possible role of androgen precursors such as DHEA as a circulating substrate for sex steroid production outside the breeding season might ensure the benefits of T such as territorial defense without the possible costs of elevated plasma T on other traits (Wingfield et al., 2001).

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