Hormones and Behavior 154 (2023) 105393

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Does capacity to produce androgens underlie variation in female ornamentation and territoriality in White-shouldered Fairywren (*Malurus alboscapulatus*)?

Jordan Boersma [a,b,c,*], Erik D. Enbody [d,e], Serena Ketaloya [e,f], Heather E. Watts [a,g], Jordan Karubian [e], Hubert Schwabl [a,g]

[a] School of Biological Sciences, Washington State University, Pullman, WA, USA
[b] Cornell Lab of Ornithology, Ithaca, NY, USA
[c] Department of Neurobiology and Behavior, Cornell University, Ithaca, NY, USA
[d] Department of Biomolecular Engineering, University of California, Santa Cruz, CA, USA
[e] Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, USA
[f] Porotona Village, Milne Bay Province, Papua New Guinea
[g] Center for Reproductive Biology, Washington State University, Pullman, WA, USA

ARTICLE INFO

Keywords:
Gonadotropin-releasing hormone
GnRH, female competition
Aggression
Simulated territorial intrusion
Social signal
Social selection
Testosterone
Challenge hypothesis

ABSTRACT

Historic bias toward study of sex hormones and sexual ornamentation in males currently constrains our perspective of hormone—behavior—phenotype relationships. Resolving how ornamented female phenotypes evolve is particularly important for understanding the diversity of social signals across taxa. Studies of both males and females in taxa with variable female phenotypes are needed to establish whether sexes share mechanisms underlying expression of signaling phenotypes and behavior. White-shouldered Fairywren (*Malurus alboscapulatus*) subspecies vary in female ornamentation, baseline circulating androgens, and response to territorial intrusion. The *moretoni* ornamented female subspecies is characterized by higher female, but lower male baseline androgens, and a stronger pair territorial response relative to pairs from the *lorentzi* unornamented female subspecies. Here we address whether subspecific differences in female ornamentation, baseline androgens, and pair territoriality are associated with ability to elevate androgens following gonadotropin releasing hormone (GnRH) challenge and in response to simulated territorial intrusion. We find that subspecies do not differ in their capacity to produce androgens in either sex following GnRH or simulated territorial intrusion (STI) challenges. STI-induced androgens were predictive of degree of response to territorial intrusions in females only, but the direction of the effect was mixed. GnRH-induced androgens did not correlate with response to simulated intruders, nor did females sampled during intrusion elevate androgens relative to flushed controls, suggesting that increased androgens are not necessary for the expression of territorial defense behaviors. Collectively, our results suggest that capacity to produce androgens does not underlie subspecific patterns of female ornamentation, territoriality, and baseline plasma androgens.

1. Introduction

Androgens mediate a variety of morphological and behavioral traits across diverse vertebrate taxa (Cox et al., 2016; Hau, 2007; Hau and Goymann, 2015). For instance, in the context of breeding, elevated androgens in male birds can cause both molt into ornamental nuptial plumage (Kimball and Ligon, 1999; Lindsay et al., 2009; Peters et al., 2000) and expression of territorial and sexual behavior at the onset of breeding (Day et al., 2006; Gleason et al., 2009; Goymann, 2009; Raouf et al., 1997; Schwabl et al., 2005; Wiley and Goldizen, 2003; Wingfield et al., 1990). These pleiotropic actions of androgens can link morphology and behavior to produce variation in integrated phenotypes (Lipshutz et al., 2019). Comparative androgen studies among individuals or populations varying phenotypically can help identify which traits might be under common androgenic control. However, t can be difficult to draw conclusions about phenotypic integration from single

* Corresponding author at: Cornell Lab of Ornithology, Ithaca, NY 14850, USA.
E-mail address: jordan.boersma@gmail.com (J. Boersma).

https://doi.org/10.1016/j.yhbeh.2023.105393
Received 15 February 2023; Received in revised form 31 May 2023; Accepted 2 June 2023
Available online 16 June 2023

blood samples (Cox et al., 2016) due to the myriad causes of rapid changes in circulating levels prior to sampling (Goymann, 2009; Oliveira, 2004). Gonadotropin-releasing hormone (GnRH) causes a trophic cascade that stimulates the gonads to produce and release androgens (Bergeon Burns et al., 2014; McGlothlin et al., 2008). Intramuscular injections of GnRH ("GnRH challenges") afford repeatable measurements of an individual's capacity to produce androgens, thus providing a reliable assessment of potential constraints on androgen-regulated signaling (Ambardar and Grindstaff, 2017; Bergeon Burns et al., 2014; Cain and Pryke, 2017; Jawor et al., 2007; McGlothlin et al., 2007).

Differences in secretion of androgens in response to GnRH challenge have been shown to correlate with integrated morphological and behavioral phenotypes in some male birds, suggesting that varying capacity to produce androgens underlies phenotype expression (Cain and Pryke, 2017; McGlothlin et al., 2008; Mills et al., 2008; Spinney et al., 2006). One recent study used GnRH challenges in two closely related coucal species differing in mating system to show that capacity to produce androgens was higher in females of the polyandrous species (Goymann, 2023), suggesting the possibility of a functional link between androgen production capacity and behaviors underlying alternate mating systems. GnRH challenge studies in females that vary in coloration are limited, with one study of female White-throated Sparrow (*Zonotrichia albicollis*) morphs showing no difference in GnRH-induced testosterone levels, despite morph-specific variation in male GnRH response in the same species (Spinney et al., 2006). Despite the commonality of female ornamentation in nature, there has been relatively little research on its mechanistic basis, leaving a gap in our understanding of the processes of signal evolution. Androgens appear to mediate female ornamentation in some polyandrous bird species (Johns, 1964; Muck and Goymann, 2011), though studies in more common mating systems are sparse (Lahaye et al., 2014; Moreno et al., 2014). The avian ovary synthesizes and secretes androgens and can be stimulated by GnRH injection to do so (Bentz et al., 2022; Egbert et al., 2013; Ketterson et al., 2005; Tilly et al., 1991), although circulating levels of androgens, including testosterone, are generally lower in females than males (Goymann and Wingfield, 2014; Ketterson et al., 2005; Møller et al., 2005). Furthermore, exogenous testosterone can stimulate molt of ornamented feathers in otherwise cryptically-colored females (Boersma et al., 2020; Lank et al., 1999; Lindsay et al., 2016). Thus, female birds of at least some species have the physiological response mechanisms (i.e. receptors and genes) to develop androgen-induced ornamentation, even when it is not developed naturally. Variable capacity to produce androgens in response to GnRH is therefore a prime candidate for explaining diversity in female ornamentation.

Until recently, social signaling traits like ornaments were understudied in females and were thought to be simply the byproduct of selection on male elaboration (Darwin, 1871; Lande, 1980). Yet female ornaments often function in the context of competition for ecological resources (i.e. social selection, Tobias et al., 2012), though they can also serve to attract mates (i.e. sexual selection, (Hare and Simmons, 2019; Tobias et al., 2012). Territorial defense is ubiquitous across avian taxa, and both sexes often defend territories jointly as breeding pairs (Burtka and Grindstaff, 2015; Cain et al., 2015). Androgens have been shown to correlate with and stimulate expression of territory defense behaviors in many male (Goymann et al., 2015; Hau and Goymann, 2015; Oliveira et al., 2009), and some female bird species (Rosvall, 2013). That androgens often increase in males during periods of enhanced territory competition led to the formulation of the challenge hypothesis, which posits that conflict with same sex rivals causes rapid elevations of androgens (Wingfield et al., 1990). Evidence for agonistic encounter induced androgen elevations is mixed (reviewed in (Goymann et al., 2019; Hirschenhauser and Oliveira, 2006), though most studies are limited to males, despite the ubiquity of pair territorial defense (Hau et al., 2000; Horton et al., 2012; Rosvall, 2013; Schwabl et al., 2005).

Here we assess whether capacity to produce androgens underlies phenotypic differences in female ornamentation and territoriality across subspecies of White-shouldered Fairywren (*Malurus alboscapulatus*). The *moretoni* subspecies of this New Guinea endemic bird species is characterized by male-like ornamentation of females and greater pair territoriality, whereas the females of the *lorentzi* subspecies are cryptic brown ('unornamented') and pairs defend territories less aggressively and show greater social connectivity (Boersma et al., 2022a; Enbody et al., 2018). In addition to differences in female ornamentation and social behavior, the subspecies also differ in their baseline circulating androgen levels. Plasma androgens are higher on average in ornamented *moretoni* females relative to unornamented *lorentzi* females (Enbody et al., 2018); however, male androgens are higher on average in *lorentzi* compared to *moretoni* despite males being similarly ornamented in both subspecies (Boersma et al., 2022a). A testosterone-implant experiment with unornamented *lorentzi* females revealed that androgens induced development of some female ornamentation (white shoulder patches), which in turn seemed to enhance territory defense (Boersma et al., 2020). Finally, an experimental study found that male androgens increased subtly in response to simulated territorial intrusions relative to flushed controls, however levels were much higher in response to simulated courtship competition (Boersma et al., 2022a).

Collectively, previous studies of White-shouldered Fairywren have shown that androgens are linked to the development of ornamentation in females and territorial and sexual behavior in males (Table 1). In the current study, we use GnRH and simulated territorial intrusion challenges to assess whether varying capacity to produce androgens underlies subspecific variation in female ornamentation, territorial behavior, and baseline androgen levels. In conjunction with a recent study of variation in male androgen levels (Boersma et al., 2022a), we also test whether simulated territorial intrusions cause females to elevate androgens relative to flushed controls ("challenge hypothesis" in females; Wingfield et al., 1990).

2. Materials and methods

2.1. Study system and general field methods

White-shouldered Fairywrens are endemic to savanna and edge habitat in New Guinea and belong to the avian passerine family Maluridae (Schodde, 1982). This species shows low levels of cooperative breeding, and pairs defend territories and can breed year-round (Enbody et al., 2018, 2019). Females alone build nests and incubate while males provision females during these stages and then assist females in feeding nestlings (Enbody et al., 2019). We studied populations belonging to the *moretoni* subspecies which contains females with contrasting black-and-white ('ornamented') plumage akin to males, and populations of the *lorentzi* subspecies, containing cryptic brown ('unornamented') females

Table 1
Comparison of subspecies in plumage ornamentation, social environment and behavior, and androgen circulation. 'Lower' and 'higher' refer to statistically significant differences, and 'no sig. diff.' reflects a lack of significant difference absent means being equivalent, and 'equivalent' refers to comparisons with statistically equivalent means. Citations are listed for each comparative study.

	Subspecies		
	M. a. lorentzi	*M. a. moretoni*	*Citation*
♀ **ornamentation**	no	yes	
♂ **ornamentation**	yes	yes	
Pop. density	lower	higher	*Enbody et al., 2019*
Sociality	higher	lower	*Boersma et al., 2022a*
Territoriality	lower	higher	*Enbody et al., 2018*
♀ **baseline androgens**	lower	higher	*Enbody et al., 2018*
♂ **baseline androgens**	higher	lower	*Boersma et al., 2022a*
♀ **GnRH-androgens**	no sig. diff.		*current study*
♂ **GnRH-androgens**	equivalent		*current study*

in Papua New Guinea. Our work with *moretoni* was conducted around the villages of Podagha (149° 90′ E, 9°69′ S, 50–60 m) and Porotona, Milne Bay Province (150° 35′ E, 10°15′ S, 10–20 m a.s.l.). We conducted work with *lorentzi* in two populations near Obo village, Western Province: one was along the western edge of the Fly River drainage (141°19′ E, 7°35′ S, 10–20 m a.s.l.), and the other was a chain of islands in the Fly River (141°16′ E, 7°34′ S, 10–20 m a.s.l.).

We captured individuals in mistnets between 6:15–11:10 and 16:16–18:00 during peaks of daily Fairywren activity from May 23 to July 24, 2019. Pairs were randomly assigned to be flushed into nets absent a social stimulus or during a simulated territorial intrusion (see full details in Section 2.2). In conjunction with a previous experiment (Boersma et al., 2022a), some *lorentzi* males ($N = 14$) were captured during extra-pair courtship competitions. We assessed breeding stage based on whether pairs had recently fledged young with them, and whether captured females had a distended abdomen indicative of egg formation/laying or had a defeathered brood patch indicative of egg incubation/nestling stage. For females with brood patches, but no distended abdomen, we used degree of vascularization and wrinkling of skin to estimate whether pairs were in the incubation or nestling stage. Pairs containing a female carrying an egg and/or exhibiting a highly vascularized brood patch were grouped in stage 1 (laying and incubating; $N = 10$ females and 9 males); pairs with fledglings and/or a female with a wrinkly brood patch were grouped in stage 2 (nestling and fledgling; $N = 6$ females and 4 males); and stage 3 contained all pairs with no signs of breeding activity ($N = 40$ females and 55 males) and cases of males captured absent their mate where we were unable to estimate stage ($N = 13$ males of unknown stage). Capture, blood sampling, GnRH injection, and behavioral protocols were all approved by the Institutional Animal Care and Use Committee (IACUC) at Washington State University (protocol #: ASAF-04573).

2.2. Experimental design

2.2.1. GnRH challenges

We collected blood samples from the jugular vein using BD 31 g insulin syringes transferred to heparinized capillary tubes for each individual at two time-points: once immediately (i.e., within 10 min) after capture in a mistnet to assess baseline androgens and again after ~30 min (range: 29.6–41.2 min, mean = 32.1 min) following administration of gonadotropin-releasing hormone (GnRH). GnRH challenges consisted of a standard dose of 500 ng of chicken GnRH-I (Bachem H-3106, Torrance, CA, USA) dissolved in 10 μl of sterile saline injected into the left pectoralis major. This dose was determined based on previous GnRH injections in a congener (Barron et al., 2015) and another similarly-sized passerine (McGlothlin et al., 2008). All birds were held in a cloth bag between baseline and GnRH-induced sample collection. We aimed for ~20 μl of plasma at both time points; baseline samples ranged from 12.1 to 32.6 μl (mean: 23.0 μl) and GnRH-induced samples from 8.4 to 33.1 μl (mean: 23.2 μl).

2.2.2. Androgens during simulated territorial intrusions

In conjunction with a companion study (Boersma et al., 2022a), pairs were captured while actively defending territories against simulated intruders to test whether baseline or GnRH-induced androgen titers explained intensity of territorial defense. We refer to baseline androgens in this specific context as STI-induced androgens hereafter. We used an established simulated territorial intrusion protocol (STI) meant to mimic territorial disputes in this species (full details in Enbody et al., 2018). Briefly, cardstock mount pairs of the focal subspecies (N = 4 exemplars for each sex) were placed within the territory of a focal pair and recorded duet song ($N = 8$ exemplars of local subspecies) was played from a nearby speaker to elicit territory defense. To assess territorial defense, we recorded latency and rate of solo songs/pair duets (combined) and flybys, which occur when an individual flies within 2 m of mounts, as well as latency and proportion of time within 5 m of mounts. We quantified these responses for 10 min, at which point we opened a furled net that was placed approx. 5 m from mounts. We did not observe any effect of the furled net on the behavior of territory defending pairs, and all individuals were captured while responding to the intrusion. The duration of playback until capture ranged from 11 to 24 min (mean = 14.83 min) for females and 11–43 min (mean = 18.43 min) for males. In all cases individuals included in this experiment had an initial blood sample taken within 10 min of capture (mean = 4.02 min for females and 4.32 min for males). Following the initial blood sample, we injected individuals with GnRH (same details as above) and collected another blood sample ~ 30 min later (range: 29.47–41.47 min).

2.2.3. Challenge hypothesis experiment in females

To test whether females elevate androgens during territorial defense, we compared females caught while defending territories (using the simulated territorial intrusion protocol described above) versus flushed controls. Flushed controls were females that were foraging on their territory absent conspecifics outside of their familial group at the time of capture. We used slow movements to guide individual pairs into mistnets to avoid eliciting a stress response. All blood samples were taken within 8 min of capture (mean = 4.15 min; range: 2.08–7.5 min).

2.3. Plasma storage and radioimmunoassay

At the end of each field day, we centrifuged blood samples at 11,000 rpm for 4 min to separate plasma from red blood cells and used a Hamilton™ syringe to transfer plasma into 0.4 ml of 200 proof ethanol (Fisher Bioreagents™) in individual Eppendorf™ tubes. Samples were stored at room temperature in the field, then transferred to a refrigerator in our lab at Washington State University. Prior to assay we randomized samples across assays to account for inter-assay variation. We used an established radioimmunoassay protocol to measure total androgen titres (Barron et al., 2013; Lantz et al., 2017; Lindsay et al., 2011). Briefly, we transferred the supernatant of plasma samples over extrelut columns (MilliporeSigma, Billerica, MA), and extracted steroids using diethyl ether. Each sample was assayed in single 100 μl aliquots after resuspension in phosphate-buffered saline with gelatin, pH 7.1 (PBSg). Total androgens are reported instead of testosterone specifically because the antibody we used (Wien Laboratories T-3003, Flanders, NJ, USA) cross-reacts strongly with 5α-dihydrotestosterone (DHT). Androgen values were determined for each sample by dividing androgen concentration by the recovery rate, dividing that value by plasma volume, and correcting for volume of extract used in assay. The minimum detectable androgen concentration for this study was 393.73 pg/ml based on a plasma volume of 9.7 μl (lowest in this study) and mean recovery rate of 65.86 %. The inter-assay coefficient of variation across 5 assays was 13.57 % (calculated following methods in Chard, 1995).

2.4. Statistical methods

All analyses were conducted in R (<www.r-project.org>) version 3.6.1. We natural log transformed all androgen values prior to analysis. We compared subspecies in response to GnRH using linear models, binomial generalized linear models, and ANOVA in base R (R Development Core Team, 2019, 2019). We used backward stepwise selection throughout to remove candidate predictors with $p \geq 0.2$ (following Wang et al., 2008). We confirmed homoscedasticity of variances and inspected Q-Q residual plots to check for approximate normality for our linear models.

2.4.1. Androgen detectability analyses

We built a binomial generalized linear model in base R to test candidate predictors of whether individuals had detectable androgens. The initial model included subspecies, breeding stage, net-to-bleed time, and time-of-day bled before removing terms with $p \geq 0.2$.

2.4.2. Response to GnRH challenges across subspecies

We first compared log transformed baseline androgens among detectable samples to test whether our female and male datasets aligned with previously published patterns of mean sex-specific androgens across subspecies (Boersma et al., 2022a; Enbody et al., 2018). Breeding stage, net-to-bleed time, and time-of-day bled were included as covariates in these models. We then built separate models for males and females comparing subspecies in three metrics of response to GnRH: a) binary response (Y/N) variable which reflects whether androgens increased from baseline to GnRH-induced sampling regardless of magnitude of increase, b) change (Δ) in log-transformed androgens from baseline to GnRH-induced sampling, and c) GnRH-induced log-transformed androgen levels. For the latter two variables, we only analyzed individuals who increased androgens following GnRH challenge as we were interested in comparing subspecies without the confound of including individuals who were physiologically incapable of responding to GnRH. Breeding stage, time of day bled, and delay time between GnRH injection and second blood sample ('GnRH-to-bleed time') were included as covariates for all three response variables. We removed three outliers (two *lorentzi* males, one *moretoni* female) from Δandrogens and GnRH-induced androgen analysis due to levels exceeding suspected physiological limits (i.e. likely the product of measurement error). For individuals captured multiple times for this experiment we took the first sample collected ($N = 1$ female and 5 males).

2.4.3. Androgen predictors of response to STI

We reduced our 5 simulated territorial intrusion response variables to 2 using principal component analysis (PCA) with the prcomp command in R, then analyzed androgen predictors of each principal component using linear models. Males and females were run in separate PCAs to allow for sex-specific differences in response, and we analyzed principal components with eigenvalues > 1.0 in separate linear mixed models by sex. After excluding non-detectable androgen samples we analyzed predictors of STI response in 12 females and 15 males sampled for STI-induced androgens, and 11 females and 21 males with detectable GnRH-induced androgens. We first tested whether playback time, time-of-day bled, net-to-bleed time (for STI-induced androgens), and GnRH-to-bleed time (for GnRH-induced androgens) affected androgen levels in these datasets. We accounted for terms with $p < 0.2$ by saving residuals from linear models with each PC as the independent variable and androgen levels as the dependent variable (following Boersma et al., 2021; Vernasco et al., 2020). In these cases we replaced androgen variables with the residuals accounting for these effects on androgen levels. We used androgen variables as described above as candidate predictors with breeding stage, subspecies, and the interaction between subspecies and both androgen variables. For models including GnRH-induced androgens we added the binary GnRH response variable to test whether capacity to produce androgens following GnRH challenge predicted STI response.

2.4.4. Challenge hypothesis test in females

We compared females captured during STIs to flushed controls using the binary (Y/N) variable reflecting androgen detectability (see Section 2.3 for details) and log-transformed androgen levels among females with detectable androgens. For both models we included breeding stage, playback time, net-to-bleed time and time-of-day bled, as well as subspecies and the interaction between subspecies and sampling context (STI vs. flush) as initial candidate predictors.

2.4.5. Equivalence tests

When group comparisons were null (i.e. GnRH-induced androgen across subspecies and baseline androgens across female sampling contexts) we used equivalence tests in R package TOSTER (Lakens, 2017) to test whether means were equivalent. We used this approach to determine whether null comparisons across groups were indicative of no meaningful difference in androgens rather than a constraint of small sample sizes obscuring our ability to detect differences. We interpreted $p < 0.05$ for these tests as evidence that means were equivalent, and thus null comparisons were reflective of a true lack of difference. Equivalence tests that resulted in $p > 0.05$ indicated that group means were not statistically equivalent, therefore lack of differences in the initial comparison might be indicative of constrained sample sizes that precluded detection of an effect. We set equivalence bounds according to mean differences across subspecies previously measured in females (Enbody et al., 2018) and males (Boersma et al., 2022a) to set bounds for each sex.

3. Results

3.1. Androgen detectability

We excluded all samples that fell below the assay detection limit from analysis of androgen levels to remove artificial variation due to variable plasma volumes ($N = 56$ of 213 total samples). Detectability differed by sex in baseline ($X^2 = 12.92$, df = 1, $p < 0.001$) and GnRH-induced samples ($X^2 = 20.10$, df = 1, $p < 0.00001$). Of baseline samples, 29 of 94 samples (31 %) samples were undetectable, 22 of which were female samples, whereas 23 % ($N = 27$ of 119 samples) of GnRH-induced samples were undetectable, again with 22 females having undetectable androgens. Subspecies did not differ in proportion of detectable samples prior to ($X^2 = 0.39$, df = 1, $p = 0.53$) or after GnRH challenge ($X^2 = 0.42$, df = 1, $p = 0.52$). Detectability differed by breeding stage in baseline ($X^2 = 7.05$, df = 2, $p = 0.03$), but not GnRH-induced samples ($X^2 = 3.40$, df = 2, $p = 0.18$) with sexes pooled. Among baseline samples, 7 of 19 (37 %) were undetectable in individuals captured during the laying or incubation stages, whereas no birds sampled during nestling and fledgling had undetectable androgens, and 23 of 95 (24 %) samples were undetectable among birds not known to be breeding.

3.2. Baseline and GnRH-induced androgens across subspecies

We analyzed baseline androgens in 34 females ($N = 14$ *lorentzi* and 20 *moretoni*) and 60 males ($N = 31$ *lorentzi* and 29 *moretoni*). Baseline androgens differed among male ($p = 0.02$), but not female ($p = 0.56$), subspecies after removing undetectable samples (Table S1). Males from the *lorentzi* subspecies had greater mean androgens (mean ± sd: 1291.24 ± 1046.27 pg/ml) than *moretoni* males (mean ± sd: 876,75 ± 734,81 pg/ml; $N = 30$ for both). Female baseline androgens were not statistically equivalent across subspecies ($t = 0.94$, $p = 0.18$; *lorentzi* mean ± sd: 829.63 ± 1096.40 pg/ml, N = 14; *moretoni* mean ± sd: 718.72 ± 498.74 pg/ml, $N = 20$).

We analyzed response to GnRH in 53 females: the proportion of females who elevated androgens in response to GnRH injection did not differ among subspecies ($p = 0.76$, Table S2a), with 5 of 22 (23 %) *lorentzi* and 9 of 31 *moretoni* (29 %) females elevating androgens following GnRH. GnRH-to-bleed time was predictive of female response to GnRH, with later times being associated with an increased probability of GnRH-induced increase in androgens ($p = 0.02$; mean = 33.35 min for female's that elevated androgens vs. 31.70 mean min for females who did not elevate androgens after challenge; Fig. S1). Breeding stage and time of day did not predict whether females elevated androgens following GnRH injection.

We analyzed Δ androgens from baseline to GnRH-induced sampling in 5 *lorentzi* and 9 *moretoni* females. Female subspecies also did not differ in Δ androgens from baseline to GnRH-induced samples (Table S2b), or log transformed GnRH-induced androgen levels (Fig. 1, Table S2c). The only significant covariate for the two continuous GnRH-induced variables in females was time-of-day bled in the Δ androgens and GnRH-induced androgen level analyses ($p = 0.048$ and $p = 0.004$, respectively; Fig. S2), with later times associated with lower androgens. Mean GnRH-induced androgens were not equivalent across female subspecies

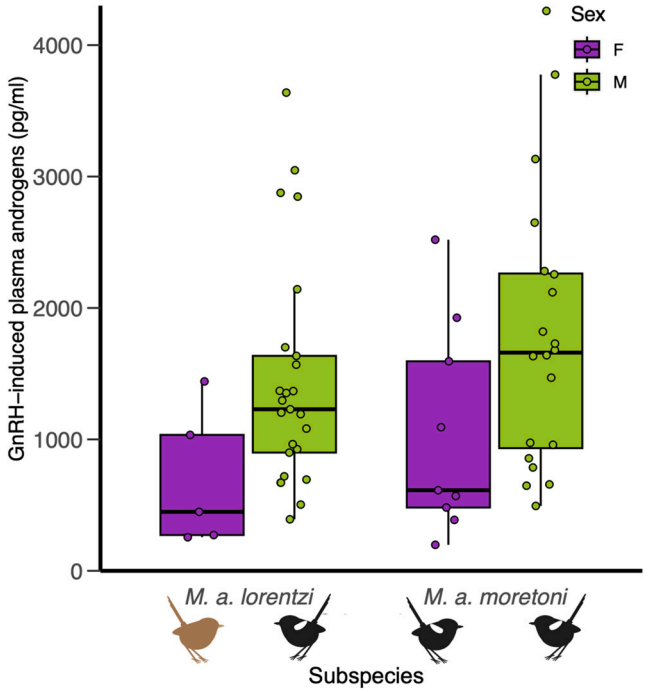

Fig. 1. Plasma androgens following GnRH challenge among subspecies (split by sex) that differ in female ornamentation. There was no difference between subspecies among females or among males. We show only individuals who elevated androgens from initial sampling following GnRH challenge.

(t = 0.16, p = 0.44; mean ± sd: 690.41 ± 525.39 pg/ml *lorentzi* vs. 1042.34 ± 801.03 pg/ml *moretoni* females), so small sample sizes could have constrained detection of subspecific variation in capacity to produce androgens.

Response to GnRH was analyzed for 66 males, with 25 of 33 (76 %) *lorentzi* and 20 of 33 (61 %) *moretoni* elevating androgens following GnRH challenge. There were no statistically significant predictors of whether males increased androgens post-GnRH, Δ androgens from baseline to post-GnRH sampling, or GnRH-induced androgen titres (Table S2; N = 45 males who increased androgens following GnRH challenge). Subspecies differed non-significantly in Δ androgens post-GnRH (p = 0.06), with *moretoni* males showing greater change in androgens from baseline to GnRH-induced sampling (Fig. S3), due to higher baseline androgens in *lorentzi* (see above) and equivalent GnRH-induced androgens across male subspecies (t = 1.86, p = 0.03; mean ± sd: 1439.64 ± 852.06 pg/ml *lorentzi* vs. 1791.08 ± 1046.89 pg/ml *moretoni* males). In other words, capacity to produce androgens is equivalent across male subspecies, so higher baseline androgens measured in *lorentzi* led to *moretoni* males showing a greater increase (Δ) from baseline to GnRH-induced sampling.

3.3. Androgen predictors of response to STI

We analyzed androgen predictors of the top 2 principal components (PCs) of STI response. We reversed the sign of both male component scores so that high scores for both components are consistent with female PCs and generally reflect greater territory defense behavior (Table 2). High PC 1 scores in both sexes reflect rapid approaches to mounts and sustained close proximity with frequent close flights past mounts, but less vocal response (i.e. longer latency and lower rate of singing/duetting). Whereas high PC 2 scores are indicative of shorter latency and more frequent songs as well as shorter latency and longer time within 5 m of mounts. After removing samples with non-detectable levels, we analyzed STI-induced samples in 12 females (5 *lorentzi* and 7 *moretoni*) and 15 males (5 *lorentzi* and 10 *moretoni*), and GnRH-induced samples in 11 females (4 *lorentzi* and 7 *moretoni*) and 17 males (6 *lorentzi* and 11 *moretoni*).

Table 2
Principal Component Analysis (PCA) parameters and variable loadings for response to simulated territorial intrusion. We analyzed the top two principal components for (a) females and (b) males separately.

(a) Female Principal Component Analysis

	PC1	PC2
Standard deviation	1.73	1.56
Proportion of Variance	34.58	31.25
Latency to 5 m	**−0.51**	**−0.76**
Latency to sing/duet	**0.58**	**−0.54**
Time within 5 m	**0.75**	**0.53**
Song/duet rate	**−0.56**	**0.55**
Flyby rate	**0.51**	−0.31

(b) Male Principal Component Analysis

	PC1	PC2
Standard deviation	1.98	1.46
Proportion of Variance	39.69	29.10
Latency to 5 m	**−0.75**	**−0.57**
Latency to sing/duet	0.44	**−0.76**
Time within 5 m	**0.83**	0.24
Song/duet rate	−0.32	**0.70**
Flyby rate	**0.67**	−0.10

Bolded terms show variable loadings of ≥ |1|.

Variation in STI-induced androgen levels was not related to playback time (p = 0.20 in females and 0.36 in males), time-of-day bled (p = 0.22 in females and 0.94 in males), or breeding stage in either sex (p = 0.51 in females and 0.60 in males), or net-to-bleed time in males (p = 0.67) so we did not include these variables in final models. We detected a trend for an effect of net-to-bleed time on STI-induced androgen levels in females (p = 0.09), so we saved residuals accounting for that effect for analysis of STI response. Likewise, we saved residuals for GnRH-induced androgens in females accounting for effects of playback time (p = 0.15), time-of-day bled (p = 0.10), and GnRH-to-bleed time (p = 0.07). Breeding stage in both sexes (p = 0.76 in females and 0.77 in males), and playback time (p = 0.76), time-of-day bled (p = 0.50) and GnRH-to-bleed time (p = 0.45) in males were not associated with GnRH-induced androgens.

The final model for PC 1 STI response (rapid and long close approach and fewer vocalizations) in 12 females with detectable STI-induced androgens included only a significant positive relationship with androgen residuals accounting for effects of net-to-bleed time (p = 0.0096; Table S4a, Fig. 2a). STI-induced androgen residuals were also a significant predictor of female PC 2 scores (quick and sustained vocal response and close proximity; p = 0.02, Table S4b), however, the relationship was negative in this case (Fig. 2b). Subspecies was a non-significant predictor of female PC 2 scores (p = 0.08), with higher PC 2 scores measured in *lorentzi* (mean ± sd score = 0.47 ± 1.58) compared to *moretoni* females (mean ± sd score = −0.49 ± 0.79). Models containing GnRH-induced androgens (N = 11) and whether females increased androgens following GnRH challenge did not produce any significant predictors of STI response (Table S4c, d).

For males, there were no significant predictors of PC 1 (rapid and sustained close approach and less vocal territoriality) or PC 2 scores (short latency and high rate of singing and rapid mount approach) in models containing detectable STI-induced (N = 15; Table S5a, b) or GnRH-induced androgen (N = 17; Table S5c, d) variables.

3.4. Test of challenge hypothesis in females

We compared androgen detectability and circulating androgen levels of 31 females captured during territorial defense and 20 flushed control

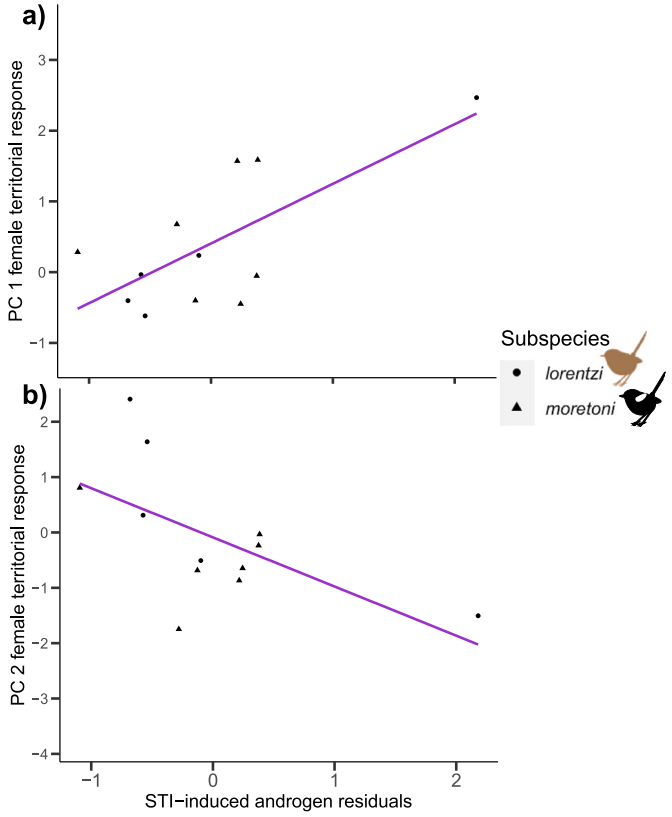

Fig. 2. Relationship between simulated intrusion principal components (PCs) and STI-induced androgen residuals (accounting for capture parameters) sampled during intrusions in females. PC 1 scores (a), which reflect rapid approaches to mounts and sustained close proximity and frequent close flights past mounts, had a positive relationship with baseline androgen residuals ($p = 0.0096$). We detected a negative relationship between baseline androgen residuals and PC 2 scores (b) in females ($p = 0.02$), which reflect shorter latency and greater rate of singing and shorter latency and longer duration of close approach to mounts. Subspecies (point shape), was a marginally non-significant predictor of PC 2 scores ($p = 0.08$).

females. The only significant predictor for whether androgens were detectable was breeding stage (Table S6a; $p = 0.006$). However, a post-hoc Tukey test did not reveal any significant differences among stages (laying/incubating vs. nestling/fledgling: $p = 1.0$, laying/incubating vs. not breeding: $p = 0.12$, nestling/fledgling vs. not breeding: $p = 1.0$). After removal of non-detectable samples, log transformed androgens did not differ among females sampled during STIs and flushed controls (Fig. 3), nor did we detect any effects of covariates (Table S5b). Female mean androgens were nearly statistically equivalent across sampling contexts ($t = 1.47$, $p = 0.07$; mean ± sd: 775.88 ± 972.57 pg/ml during STI vs. 524.37 ± 250.64 pg/ml flushed; N = 11 and 18, respectively).

4. Discussion

We tested whether differential capacity to produce androgens following GnRH challenge underlies previously found differences of mean baseline circulating androgen levels, female ornamentation, and territoriality across subspecies of White-shouldered Fairywren (Table 1). Baseline androgens were previously observed to be higher on average in females of the mutually-ornamented and more territorial *moretoni* subspecies relative to unornamented *lorentzi* females (Enbody et al., 2018), whereas male androgens were highest in the more social *lorentzi*, despite a lack of difference in male ornamentation (Boersma et al., 2022a). In the current study, baseline androgens differed among male subspecies, with the highest levels measured in *lorentzi* males as in Boersma et al.

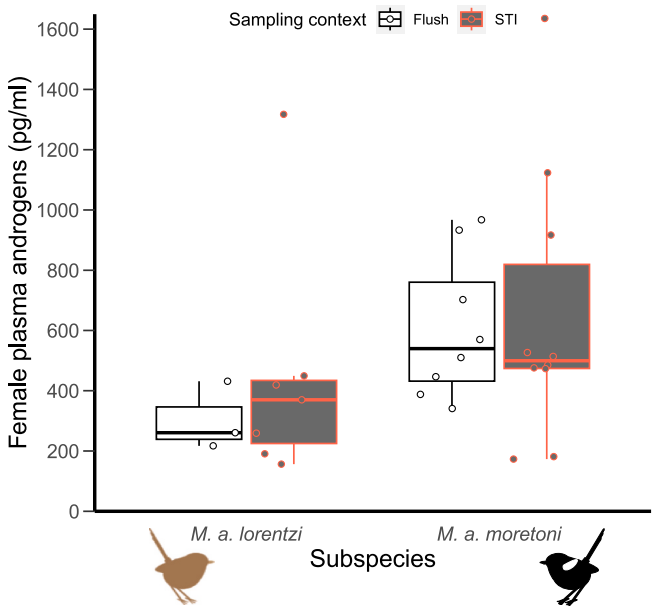

Fig. 3. Plasma androgens among females of two subspecies varying in ornamentation sampled by flushing or while responding to a simulated territorial intruder (STI). There were no differences between sampling contexts or subspecies. One *lorentzi* female sample during STI not shown due to falling out of plot area (4297.58 pg/ml).

(2022a). Subspecific variation in male baseline androgens is apparently not the product of differential capacity to produce androgens, as our present results demonstrates equivalent GnRH-induced androgen levels among male subspecies. We suggest that greater androgen levels measured in *lorentzi* males are instead the product of more frequent interactions with extra-pair females, as our previous work has shown these interactions are associated with a strong elevation of androgens in this species (Boersma et al., 2022a).

Previous work showing greater baseline androgens in ornamented *moretoni* females (Enbody et al., 2018) coupled with the finding that ornamentation can be induced with testosterone implantation in unornamented *lorentzi* females (Boersma et al., 2020) suggests that variation in female ornamentation could arise through differential capacity to produce androgens. Neither baseline or GnRH-induced androgens differed among female subspecies in our study, however, androgens were not equivalent among female subspecies in either baseline or GnRH-induced sample comparisons, so it's possible that constrained sample sizes precluded detection of subspecific variation in baseline or GnRH-induced androgens.

Collectively, our null GnRH results and previous testosterone manipulation work might suggest that the lack of ornamentation observed in *lorentzi* females could be a result of constraints on androgen secretion —despite similar capacity to produce androgens— that prevent elevation of androgens to the greater levels measured on average in *moretoni* (Enbody et al., 2018). If androgen secretion is constrained in *lorentzi* females, then we would expect that *lorentzi* females would show reduced androgenic response to social challenges like simulated territorial intrusions. However, female subspecies did not differ in their androgen response to simulated intrusion, with neither elevating androgens relative to flushed controls (Fig. 3). Mean androgens were nearly statistically equivalent across sampling groups, suggesting the lack of effect was biologically meaningful rather than an artifact of constrained sample sizes. It is possible that female plumage variation is the result of differences in response to circulating androgens across subspecies (e.g. receptors, enzymes, transcription factors, etc. (Bergeon Burns et al., 2014; Béziers et al., 2017; Fuxjager et al., 2013; Hau and Goymann, 2015; Rosvall et al., 2012; Sewall, 2015; Soma et al., 2003).

Though a study of gene expression in developing feather follicles of female White-shouldered Fairywrens found that these subspecies do not differ in the expression of androgen (or estrogen) receptors (Enbody et al., 2022); receptor protein abundance (Lizotte et al., 2009) or expression may differ in other tissues.

Androgens like testosterone are known to mediate expression and degree of territorial behavior in males across diverse taxa (Hau et al., 2000; Oliveira et al., 2009; Pärn et al., 2008; Sandell, 2007; Sperry et al., 2010), and androgens can vary with territoriality in females (Cain and Ketterson, 2012, 2013; Kriner and Schwabl, 1991; Pärn et al., 2008; Rosvall, 2013). In our study species, territory density and defense are higher on average in both sexes of *moretoni* relative to *lorentzi* (Enbody et al., 2018, 2019). We assessed whether individual and subspecific variation in response to simulated territorial intrusions were associated with higher STI-induced and GnRH-induced androgens, which would suggest a functional link between androgen secretion and/or capacity for increase and territoriality. STI-induced androgens were correlated with degree of territoriality in females, though the direction of the effect was mixed as we found a positive association with the first, and negative association with the second principal component of intrusion responses (Fig. 2). These results indicate that STI-induced androgens had a positive association with close flights past mounts, a negative relationship with vocal territoriality, and an equivocal relationship with close approach to mounts based on variable loadings on each component (Table 2). In males, STI-induced androgens did not correlate with response to simulated intrusion. Collectively, we do not find evidence for elevated circulating androgens being a prerequisite for territory defense in this species. Possibly, 10 min of playback time was insufficient for causing elevated androgens in this species, as a study of another tropical bird found 120 min was required to elevate androgens (Wikelski et al., 1999). Though we did not find a relationship between playback time and androgen elevation in our experiment (Boersma et al., 2022a), all individuals were captured within an hour of playback starting.

No metric of response to GnRH challenge explained response to simulated territorial intrusions in either sex. Taken with the result that females did not substantially elevate androgens during territorial intrusions and a complementary study showing only marginally higher androgens in males during intrusions (Boersma et al., 2022a), we do not find that territory defense is contingent upon the capacity to produce androgens in either sex. As with plumage ornamentation, the greater territory defense in *moretoni* could be maintained and modulated by androgen receptor densities (Hau et al., 2004; Rosvall, 2013; Rosvall et al., 2012; Wingfield et al., 2001), or (unlike plumage) other sex steroids like progesterone (Adreani and Mentesana, 2018; Goymann et al., 2008), estradiol (Pärn et al., 2008; Soma et al., 2000, 2008), or dehydroepiandrosterone (DHEA; Soma et al., 2002, 2015). These alternative mechanisms could allow maintenance of competitive behavior absent costs of sustained androgen elevation (Wingfield et al., 2001).

Both subspecies of White-shouldered Fairywren tested in this study breed opportunistically, with both sexes showing breeding readiness year-round except for in cases of extreme drought (Boersma et al., 2022b; Enbody et al., 2019). Whereas most males responded to GnRH challenges by elevating androgens in relation to their baseline sample ($N = 45$ of 66 males, 68 %), comparatively fewer females elevated androgens following GnRH challenge ($N = 14$ of 53 females, 26 %). The low percentage of response to GnRH may suggest that many females, in contrast to males, reduce gonadal endocrine activity when they are not breeding. In Dark-eyed Junco (*Junco hyemalis*), the largest magnitude of increase in androgens following GnRH challenge occurred in early breeding in males (Jawor et al., 2006), while females only elevated androgens in response to GnRH challenge in the 7 days prior to oviposition (Jawor et al., 2007). George and Rosvall (2018) found a similar result in female Tree Swallows (*Tachycineta bicolor*), as androgens were only elevated following GnRH injection during territory establishment and pre-lay. In a congener to our study species, females injected during incubation and nestling stages were unresponsive to GnRH challenge (Lindsay et al., 2016), despite males increasing androgens following injection during the same experiment (Barron et al., 2015). Though we did not detect a clear effect of breeding stage on whether a female elevated androgens following GnRH challenge, we used a coarse assessment of breeding stage based upon presence/absence of fledglings on territories and whether females were yolking eggs or had highly vascularized brood patches. Therefore, a lack of effect of breeding stage on GnRH response should not be taken as conclusive evidence that capacity to produce androgens does not differ across breeding stages, as has been shown in other species (George and Rosvall, 2018; Jawor et al., 2007).

As a final consideration, we measured androgens in blood drawn from the jugular vein, which is thought to reflect steroid metabolism by the brain and brain-derived steroids (Newman et al., 2008; Saldanha and Schlinger, 1997; Soma et al., 2008). One might speculate that we failed to measure androgens produced by the gonads in response to GnRH challenge. However, given that greater than two thirds of males increased androgens following injection, and a previous study that found consistent elevation of androgens in males using the same GnRH and blood sampling protocol in a congener (Barron et al., 2015), it seems unlikely that our jugular samples failed to capture gonad-derived androgens in response to GnRH. Whereas we did not include a vehicle-injected control group for our GnRH experiment, androgen concentrations typically decline in males following capture and handling (Lindsay, 2010; Vernasco et al., 2019; Woodley and Lacy, 2010) and the increases we measured in GnRH-induced samples are likely to reflect natural variation in capacity to produce androgens.

5. Conclusions

In our study of White-shouldered Fairywrens, females of the two subspecies that differ in baseline circulating androgens, territoriality, and ornamentation did not differ in androgen response to either GnRH or simulated territorial intrusion challenges. Higher baseline androgens in males of the unornamented female *lorentzi* subspecies (this study; Boersma et al., 2022a) coupled with the absence of a difference in male androgen response to GnRH challenge across subspecies suggest that the ornamented female phenotype is not the correlated product of selection on androgen production in males. Collectively, studies of this species suggest that androgens have a role in inducing female plumage ornamentation and some aspects of female territory defense behavior in the ornamented phenotype (Boersma et al., 2020; Enbody et al., 2018). Future work on androgen response pathways (i.e., receptors and enzymes in different target tissues) could resolve the full extent to which androgens mediate variation in female ornamentation and behavior in this species. Determining the mechanisms underlying variation in female phenotype such as ornamentation and behavior will greatly improve our understanding how phenotypes evolve across diverse taxa.

Data availability

Data will be made available on request.

Acknowledgements

This work wouldn't have been possible without the support of traditional landowners at each of our field sites in Papua New Guinea. Many individuals assisted with fieldwork, and we'd like to thank J. A. Gregg, D. Nason, M. Olesai, J. Sarusaruna, John, Kipling, Kingsford, and Manu for their expert help in the field.

Funding

This work was supported by funds from Richard and Nancy Mack and the National Science Foundation (IOS-354133 to J. K. and IOS-1352885 to H. S.).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2023.105393.

References

Adreani, N.M., Mentesana, L., 2018. Not One Hormone or Another: Aggression Differentially Affects Progesterone and Testosterone in a South American Ovenbird Not One Hormone or Another: Aggression Differentially Affects Progesterone and Testosterone in a South American Ovenbird, pp. 0–28. https://doi.org/10.1101/371690.

Ambardar, M., Grindstaff, J., 2017. Pre-GnRHBaseline and GnRH-induced testosterone levels do not vary across behavioral contexts: a role for individual variation. Gen. Comp. Endocrinol. 246, 51–62. https://doi.org/10.1016/j.ygcen.2017.03.009.

Barron, D.G., Webster, M.S., Schwabl, H., 2013. Body condition influences sexual signal expression independent of circulating androgens in male red-backed fairy-wrens. Gen. Comp. Endocrinol. 183, 38–43. https://doi.org/10.1016/j.ygcen.2012.12.005.

Barron, D.G., Webster, M.S., Schwabl, H., 2015. Do androgens link morphology and behaviour to produce phenotype-specific behavioural strategies? Anim. Behav. 100, 116–124. https://doi.org/10.1016/j.anbehav.2014.11.016.

Bentz, A.B., Empson, T.A., George, E.M., Rusch, D.B., Buechlein, A., Rosvall, K.A., 2022. Hormones and Behavior How Experimental Competition Changes Ovarian Gene Activity in Free-Living Birds: Implications for Steroidogenesis, Maternal Effects, and Beyond, p. 142.

Bergeon Burns, C.M., Rosvall, K.A., Hahn, T.P., Demas, G.E., Ketterson, E.D., 2014. Examining sources of variation in HPG axis function among individuals and populations of the dark-eyed junco. Horm. Behav. 65, 179–187. https://doi.org/10.1016/j.yhbeh.2013.10.006.

Béziers, P., Ducrest, A.L., Simon, C., Roulin, A., 2017. Circulating testosterone and feather-gene expression of receptors and metabolic enzymes in relation to melanin-based colouration in the barn owl. Gen. Comp. Endocrinol. 250, 36–45. https://doi.org/10.1016/j.ygcen.2017.04.015.

Boersma, J., Enbody, E.D., Jones, J.A., Nason, D., Lopez-Contreras, E., Karubian, J., Schwabl, H., 2020. Testosterone induces plumage ornamentation followed by enhanced territoriality in a female songbird. Behav. Ecol. 31, 1233–1241. https://doi.org/10.1093/beheco/araa077.

Boersma, J., Barron, D.G., Baldassarre, D.T., Webster, M.S., Schwabl, H., 2021. Wildfire affects expression of male sexual plumage through suppressed testosterone circulation in a tropical songbird. J. Avian Biol. 52 https://doi.org/10.1111/jav.02757.

Boersma, J., Anthony, J., Enbody, E.D., Welklin, J.F., Ketaloya, S., Nason, D., Karubian, J., Schwabl, H., 2022a. Male White-shouldered Fairywrens (Malurus alboscapulatus) elevate androgens greater when courting females than during territorial challenges. Horm. Behav. 142, 105158 https://doi.org/10.1016/j.yhbeh.2022.105158.

Boersma, J., Enbody, E.D., Karubian, J., Watts, H.E., Schwabl, H., 2022b. Drought disrupts year-round breeding readiness in a tropical songbird. Avian Conservation and Ecology 17. https://doi.org/10.5751/ACE-2343-170244.

Burtka, J.L., Grindstaff, J.L., 2015. Similar nest defence strategies within pairs increase reproductive success in the eastern bluebird, Sialia sialis. Anim. Behav. 100, 174–182. https://doi.org/10.1016/j.anbehav.2014.12.004.

Cain, K.E., Ketterson, E.D., 2012. Competitive females are successful females; phenotype, mechanism, and selection in a common songbird. Behav. Ecol. Sociobiol. 66, 241–252. https://doi.org/10.1007/s00265-011-1272-5.

Cain, K.E., Ketterson, E.D., 2013. Costs and benefits of competitive traits in females: aggression, maternal care and reproductive success. PLoS One 8. https://doi.org/10.1371/journal.pone.0077816.

Cain, K.E., Pryke, S.R., 2017. Testosterone production in response to exogenous gonadotropin releasing hormone (GnRH challenge) depends on social environment and color polymorphism. Gen. Comp. Endocrinol. 244, 77–85. https://doi.org/10.1016/j.ygcen.2015.12.029.

Cain, K.E., Cockburn, A., Langmore, N., 2015. Female song rates in response to simulated intruder are positively related to reproductive success. Front. Ecol. Evol. 3, 119. https://doi.org/10.3389/fevo.2015.00119.

Chard, T., 1995. An Introduction to Radioimmunoassay and Related Techniques. Laboratory Techniques in Biochemistry and Molecular Biology. Elsevier, Oxford.

Cox, R.M., McGlothlin, J.W., Bonier, F., 2016. Hormones as mediators of phenotypic and genetic integration: an evolutionary genetics approach. Integr. Comp. Biol. 56, 126–137. https://doi.org/10.1093/icb/icw033.

Darwin, C., 1871. The Descent of Man, and Selection in Relation to Sex, the Descent of Man, and Selection in Relation to Sex. Murray, London.

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. https://doi.org/10.1016/j.yhbeh.2005.07.006.

Egbert, J.R., Jackson, M.F., Rodgers, B.D., Schwabl, H., 2013. Between-female variation in house sparrow yolk testosterone concentration is negatively associated with CYP19A1 (aromatase) mRNA expression in ovarian follicles. Gen. Comp. Endocrinol. 183, 53–62. https://doi.org/10.1016/j.ygcen.2012.12.001.

Enbody, E.D., Boersma, J., Schwabl, H., Karubian, J., 2018. Female ornamentation is associated with elevated aggression and testosterone in a tropical songbird. Behav. Ecol. 29, 1056–1066. https://doi.org/10.1093/beheco/ary079.

Enbody, E.D., Boersma, J., Jones, J.A., Chatfield, M.W.H., Ketaloya, S., Nason, D., Baldassarre, D.T., Hazlehurst, J., Gowen, O., Schwabl, H8., Karubian, J., 2019. Social organisation and breeding biology of the White-shouldered Fairywren (Malurus alboscapulatus). Emu 119, 274–285. https://doi.org/10.1080/01584197.2019.1595663.

Enbody, E.D., Sin, S.Y.W., Boersma, J., Edwards, S.V., Ketaloya, S., Schwabl, H., Webster, M.S., Karubian, J., 2022. The evolutionary history and mechanistic basis of female ornamentation in a tropical songbird. Evolution (N Y). https://doi.org/10.1111/evo.14545.

Fuxjager, M.J., Longpre, K.M., Chew, J.G., Fusani, L., Schlinger, B.A., 2013. Peripheral androgen receptors sustain the acrobatics and fine motor skill of elaborate male courtship. Endocrinology 154, 3168–3177.

George, E.M., Rosvall, K.A., 2018. Testosterone production and social environment vary with breeding stage in a competitive female songbird. Horm. Behav. 103, 28–35. https://doi.org/10.1016/j.yhbeh.2018.05.015.

Gleason, E.D., Fuxjager, M.J., Oyegbile, T.O., Marler, C.A., 2009. Testosterone release and social context: when it occurs and why. Front. Neuroendocrinol. 30, 460–469. https://doi.org/10.1016/j.yfrne.2009.04.009.

Goymann, W., 2009. Social modulation of androgens in male birds. Gen. Comp. Endocrinol. 163, 149–157. https://doi.org/10.1016/j.ygcen.2008.11.027.

Goymann, W., 2023. Androgen-armoured amazons: reversed sex roles in coucals are associated with testosterone in females but not males. Proc. R. Soc. B Biol. Sci. 290, 20222401. https://doi.org/10.1098/rspb.2022.2401.

Goymann, W., Wingfield, J.C., 2014. Male-to-Female Testosterone Ratios, Dimorphism, and Life History — What Does It Really Tell Us?, vol. 25, pp. 685–699. https://doi.org/10.1093/beheco/aru019.

Goymann, W., Wittenzellner, A., Schwabl, I., Makomba, M., 2008. Progesterone modulates aggression in sex-role reversed female African black coucals. Proc. R. Soc. B Biol. Sci. 275, 1053–1060. https://doi.org/10.1098/rspb.2007.1707.

Goymann, W., Villavicencio, C.P., Apfelbeck, B., 2015. Does a short-term increase in testosterone affect the intensity or persistence of territorial aggression? - an approach using an individual's hormonal reactive scope to study hormonal effects on behavior. Physiol. Behav. 149, 310–316. https://doi.org/10.1016/j.physbeh.2015.06.029.

Goymann, W., Moore, I.T., Oliveira, R.F., 2019. Challenge hypothesis 2.0: a fresh look at an established idea. Bioscience 69, 432–442. https://doi.org/10.1093/biosci/biz041.

Hare, R.M., Simmons, L.W., 2019. Sexual selection and its evolutionary consequences in female animals. Biol. Rev. 94, 929–956. https://doi.org/10.1111/brv.12484.

Hau, M., 2007. Regulation of Male Traits by Testosterone: Implications for the Evolution of Vertebrate Life Histories, pp. 133–144. https://doi.org/10.1002/bies.20524.

Hau, M., Goymann, W., 2015. Endocrine mechanisms, behavioral phenotypes and plasticity: known relationships and open questions. Front. Zool. 12, S7. https://doi.org/10.1186/1742-9994-12-S1-S7.

Hau, M., Wikelski, M., Soma, K.K., Wingfield, J.C., 2000. Testosterone and Year-Round Territorial Aggression in a Tropical Bird, vol. 33, pp. 20–33. https://doi.org/10.1006/gcen.1999.7390.

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. https://doi.org/10.1016/j.yhbeh.2003.08.002.

Hirschenhauser, K., Oliveira, R.F., 2006. Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. Anim. Behav. 71, 265–277. https://doi.org/10.1016/j.anbehav.2005.04.014.

Horton, B.M., Hauber, M.E., Maney, D.L., 2012. Morph matters: aggression bias in a polymorphic sparrow. PLoS One 7, 1–5. https://doi.org/10.1371/journal.pone.0048705.

Jawor, J.M., McGlothlin, J.W., Casto, J.M., Greives, T.J., Snajdr, E.A., Bentley, G.E., Ketterson, E.D., 2006. Seasonal and individual variation in response to GnRH challenge in male dark-eyed juncos (Junco hyemalis). Gen. Comp. Endocrinol. 149, 182–189. https://doi.org/10.1016/j.ygcen.2006.05.013.

Jawor, J.M., Mcglothlin, J.W., Casto, J.M., Greives, T.J., Snajdr, E.A., Bentley, G.E., Ketterson, E.D., 2007. Testosterone response to GnRH in a female songbird varies with stage of reproduction: implications for adult behaviour and maternal effects. Funct. Ecol. 21, 767–775. https://doi.org/10.1111/j.1365-2435.2007.01280.x.

Johns, J.E., 1964. Testosterone-induced nuptial feathers in phalaropes. Condor 66, 449–455.

Ketterson, E.D., Nolan, V., Sandell, M., 2005. Testosterone in females: mediator of adaptive traits, constraint on sexual dimorphism, or both? Am. Nat. 166, S85–S98.

Kimball, R.T., Ligon, J.D., 1999. Evolution of avian plumage dichromatism from a proximate perspective. Am. Nat. 154, 182–193. https://doi.org/10.1086/303228.

Kriner, E., Schwabl, H., 1991. Control of winter song and territorial aggression of female robins (Erithacus rubecula) by testosterone. Ethology 87, 37–44. https://doi.org/10.1111/j.1439-0310.1991.tb01186.x.

Lahaye, S.E.P., Eens, M., Darras, V.M., Pinxten, R., 2014. Bare-part color in female budgerigars changes from brown to structural blue following testosterone treatment but is not strongly masculinized. PLoS One 9, e86849. https://doi.org/10.1371/journal.pone.0086849.

Lakens, D., 2017. Equivalence tests: a practical primer for t tests, correlations, and meta-analyses. Soc. Psychol. Personal. Sci. 8, 355–362. https://doi.org/10.1177/1948550617697177.

Lande, R., 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution (N Y) 34, 292–305.

Lank, D.B., Coupe, M., Wynne-edwards, K.E., 1999. Testosterone-induced male traits in female ruffs (Philomachus pugnax): autosomal inheritance and gender differentiation. Proceeding of the Royal Society B 266, 2323–2330.

Lantz, S.M., Boersma, J., Schwabl, H., Karubian, J., 2017. Early-moulting Red-backed Fairywren males acquire ornamented plumage in the absence of elevated androgens. Emu - Austral Ornithology 117, 170–180. https://doi.org/10.1080/01584197.2017.1297206.

Lindsay, W.R., 2010. Hormonal Regulation of Male Reproductive Phenotype in a Cooperatively Breeding Tropical Bird. Washington State University.

Lindsay, W.R., Webster, M.S., Varian, C.W., Schwabl, H., 2009. Plumage colour acquisition and behaviour are associated with androgens in a phenotypically plastic tropical bird. Anim. Behav. 77, 1525–1532. https://doi.org/10.1016/j.anbehav.2009.02.027.

Lindsay, W.R., Webster, M.S., Schwabl, H., 2011. Sexually selected male plumage color is testosterone dependent in a tropical passerine bird, the red-backed fairy-wren (Malurus melanocephalus). PLoS One 6, e26067. https://doi.org/10.1371/journal.pone.0026067.

Lindsay, W.R., Barron, D.G., Webster, M.S., Schwabl, H., 2016. Testosterone activates sexual dimorphism including male-typical carotenoid but not melanin plumage pigmentation in a female bird. J. Exp. Biol. 219, 3091–3099. https://doi.org/10.1242/jeb.135384.

Lipshutz, S.E., George, E.M., Bentz, A.B., Rosvall, K.A., 2019. Evaluating testosterone as a phenotypic integrator: from tissues to individuals to species. Mol. Cell. Endocrinol. 496, 110531 https://doi.org/10.1016/j.mce.2019.110531.

Lizotte, E., Grandy, S.A., Tremblay, A., Allen, B.G., Fiset, C., 2009. Expression, distribution and regulation of sex steroid hormone receptors in mouse heart. Cell Physiology and Biochemistry 23, 75–86.

McGlothlin, J.W., Jawor, J.M., Ketterson, E.D., 2007. Natural variation in a testosterone-mediated trade-off between mating effort and parental effort. Am. Nat. 170, 864–875. https://doi.org/10.1086/522838.

McGlothlin, J.W., Jawor, J.M., Greives, T.J., Casto, J.M., Phillips, J.L., Ketterson, E.D., 2008. Hormones and honest signals: males with larger ornaments elevate testosterone more when challenged. J. Evol. Biol. 21, 39–48. https://doi.org/10.1111/j.1420-9101.2007.01471.x.

Mills, S.C., Hazard, L., Lancaster, L., Mappes, T., Miles, D., Oksanen, T.A., Sinervo, B., 2008. Gonadotropin hormone modulation of testosterone, immune function, performance, and behavioral trade-offs among male morphs of the lizard Uta stansburiana. Am. Nat. 171, 339–357. https://doi.org/10.1086/527520.

Møller, A.P., Garamszegi, L.Z., Gil, D., Hurtrez-Boussè, S., Eens, M., 2005. Correlated evolution of male and female testosterone profiles in birds and its consequences. Behav. Ecol. Sociobiol. 58, 534–544. https://doi.org/10.1007/s00265-005-0962-2.

Moreno, J., Gil, D., Cantarero, A., López-Arrabé, J., 2014. Extent of a white plumage patch covaries with testosterone levels in female Pied Flycatchers Ficedula hypoleuca. J. Ornithol. 155, 639–648. https://doi.org/10.1007/s10336-014-1046-8.

Muck, C., Goymann, W., 2011. Throat Patch Size and Darkness Covaries With Testosterone in Females of a Sex-Role Reversed Species. https://doi.org/10.1093/beheco/arr133.

Newman, A.E.M., Pradhan, D.S., Soma, K.K., 2008. Dehydroepiandrosterone and corticosterone are regulated by season and acute stress in a wild songbird: jugular versus brachial plasma. Endocrinology 149, 2537–2545. https://doi.org/10.1210/en.2007-1363.

Oliveira, R.F., 2004. Social modulation of androgens in vertebrates: mechanisms and function. Adv. Study Behav. https://doi.org/10.1016/S0065-3454(04)34005-2.

Oliveira, R.F., Silva, A., Cana, A.V.M., 2009. Why Do Winners Keep Winning? Androgen Mediation of Winner But Not Loser Effects in Cichlid Fish, pp. 2249–2256. https://doi.org/10.1098/rspb.2009.0132.

Pärn, H., Lindström, K.M., Sandell, M., Amundsen, T., 2008. Female aggressive response and hormonal correlates - an intrusion experiment in a free-living passerine. Behav. Ecol. Sociobiol. 62, 1665–1677. https://doi.org/10.1007/s00265-008-0595-3.

Peters, A., Astheimer, L.B., Boland, C.R.J., Cockburn, A., 2000. Testosterone is involved in acquisition and maintenance of sexually selected male plumage in superb fairy-wrens, Malurus cyaneus. Behav. Ecol. Sociobiol. 47, 438–445. https://doi.org/10.1007/s002650050688.

R Development Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Raouf, S.A., Parker, P.G., Ketterson, E.D., Nolan Jr., V., Ziegenfus, C., 1997. Testosterone affects reproductive success by influencing extra-pair fertilizations in male dark-eyed juncos (Aves: Junco hyemalis). Proc. R. Soc. B Biol. Sci. 264, 1599–1603. https://doi.org/10.1098/rspb.1997.0223.

Rosvall, K.A., 2013. Proximate perspectives on the evolution of female aggression: good for the gander, good for the goose? Philosophical Transactions of the Royal Society B: Biological Sciences 368, 20130083. https://doi.org/10.1098/rstb.2013.0083.

Rosvall, K.A., Bergeon Burns, C.M., Barske, J., Goodson, J.L., Schlinger, B.A., Sengelaub, D.R., Ketterson, E.D., 2012. Neural sensitivity to sex steroids predicts individual differences in aggression: implications for behavioural evolution. Proc. R. Soc. B Biol. Sci. 279, 3547–3555. https://doi.org/10.1098/rspb.2012.0442.

Saldanha, C.J., Schlinger, B.A., 1997. Estrogen synthesis and secretion in the brown-headed cowbird (Molothrus ater). Gen. Comp. Endocrinol. 105, 390–401. https://doi.org/10.1006/gcen.1996.6841.

Sandell, Maria I., 2007. Exogenous testosterone increases female aggression in the European starling (Sturnus vulgaris). Behav. Ecol. Sociobiol. 62, 255–262. https://doi.org/10.1007/s00265-007-0460-9.

Schodde, R., 1982. The Fairy-Wrens: A Monograph of the Maluridae. Landsdowne Editions, Melbourne.

Schwabl, H., Flinks, H., Gwinner, E., 2005. Testosterone, Reproductive Stage, and Territorial Behavior of Male and Female European Stonechats *Saxicola torquata*, vol. 47, pp. 503–512. https://doi.org/10.1016/j.yhbeh.2004.08.003.

Sewall, K.B., 2015. Androgen receptor expression could contribute to the honesty of a sexual signal and be the basis of species differences in courtship displays. Funct. Ecol. 29, 1111–1113. https://doi.org/10.1111/1365-2435.12498.

Soma, K.K., Tramontin, A.D., Wingfield, J.C., 2000. Oestrogen regulates male aggression in the non-breeding season. Proceedings. Biological Sciences/The Royal Society 267, 1089–1096. https://doi.org/10.1098/rspb.2000.1113.

Soma, K.K., Wissman, A.M., Brenowitz, E.A., Wingfield, J.C., 2002. Dehydroepiandrosterone (DHEA) increases territorial song and the size of an associated brain region in a male songbird. Horm. Behav. 41, 203–212. https://doi.org/10.1006/hbeh.2001.1750.

Soma, K.K., Schlinger, B.A., Wingfield, J.C., Saldanha, C.J., 2003. Brain aromatase, 5α-reductase, and 5??-reductase change seasonally in wild male song sparrows: relationship to aggressive and sexual behavior. J. Neurobiol. 56, 209–221. https://doi.org/10.1002/neu.10225.

Soma, K.K., Scotti, M.A.L., Newman, A.E.M., Charlier, T.D., Demas, G.E., 2008. Novel mechanisms for neuroendocrine regulation of aggression. Front. Neuroendocrinol. 29, 476–489. https://doi.org/10.1016/j.yfrne.2007.12.003.

Soma, K.K., Rendon, N.M., Boonstra, R., Albers, H.E., Demas, G.E., 2015. DHEA effects on brain and behavior: insights from comparative studies of aggression. Journal of Steroid Biochemistry & Molecular Biology Journal of Steroid Biochemistry and Molecular Biology 145, 261–272. https://doi.org/10.1016/j.jsbmb.2014.05.011.

Sperry, T.S., Wacker, D.W., Wingfield, J.C., 2010. The role of androgen receptors in regulating territorial aggression in male song sparrows. Horm. Behav. 57, 86–95. https://doi.org/10.1016/j.yhbeh.2009.09.015.

Spinney, L.H., Bentley, G.E., Hau, M., 2006. Endocrine correlates of alternative phenotypes in the white-throated sparrow (Zonotrichia albicollis). Horm. Behav. 50, 762–771. https://doi.org/10.1016/j.yhbeh.2006.06.034.

Tilly, J.L., Kowalski, K.I., Johnson, A.L., 1991. Stage of ovarian follicular development associated with the initiation of steroidogenic competence in avian granulosa cells. Biol. Reprod. 44, 305–314. https://doi.org/10.1095/biolreprod44.2.305.

Tobias, J.A., Montgomerie, R., Lyon, B.E., 2012. The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. Philosophical Transactions of the Royal Society B: Biological Sciences 367, 2274–2293. https://doi.org/10.1098/rstb.2011.0280.

Vernasco, B.J., Horton, B.M., Ryder, T.B., Moore, I.T., 2019. Sampling baseline androgens in free-living passerines: methodological considerations and solutions. Gen. Comp. Endocrinol. 273, 202–208. https://doi.org/10.1016/j.ygcen.2018.07.017.

Vernasco, B.J., Horton, B.M., Moore, I.T., Brandt Ryder, T., 2020. Reduced cooperative behavior as a cost of high testosterone in a lekking passerine bird. Behav. Ecol. 31, 401–410. https://doi.org/10.1093/BEHECO/ARZ201.

Wang, Q., Koval, J.J., Mills, C.A., Lee, K.I.D., 2008. Determination of the selection statistics and best significance level in backward stepwise logistic regression. Commun Stat Simul Comput 37, 62–72. https://doi.org/10.1080/03610910701723625.

Wikelski, M., Hau, M., Wingfield, J.C., 1999. Social Instability Increases Plasma Testosterone in a Year-Round Territorial Neotropical Bird.

Wiley, C.J., Goldizen, A.W., 2003. Testosterone is correlated with courtship but not aggression in the tropical buff-banded rail, Gallirallus philippensis. Horm. Behav. 43, 554–560. https://doi.org/10.1016/S0018-506X(03)00066-7.

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. https://doi.org/10.1086/285134.

Wingfield, J.C., Lynn, S.E., Soma, K.K., 2001. Avoiding the "costs" of testosterone: ecological bases of hormone-behavior interactions. Brain Behav. Evol. 57, 239–251. https://doi.org/10.1159/000047243.

Woodley, S.K., Lacy, E.L., 2010. An acute stressor alters steroid hormone levels and activity but not sexual behavior in male and female Ocoee salamanders (Desmognathus ocoee). Horm. Behav. 58, 427–432. https://doi.org/10.1016/j.yhbeh.2010.05.011.