ORIGINAL ARTICLE

Female ornamentation does not predict aggression in a tropical songbird

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Abstract

Although conflict is often adaptive and necessary to secure limited resources, it is also frequently a costly endeavor. Signals that reliably communicate competitive ability are commonly employed by animals to reduce conflict costs. Both male and female signals have the capacity to serve as honest indicators of competitive ability, but the extent to which this occurs in females has received relatively limited attention. We studied how natural feather coloration and experimentally manipulated plumage ornamentation in female white-shouldered fairywrens (*Malurus alboscapulatus moretoni*) relates to their aggressive behavior. These fairywrens are a useful study system in that female, but not male coloration varies throughout New Guinea. We tested behavioral responses to simulated rivals both prior to and after plumage manipulation via two distinct behavioral assays: simulated territorial intrusions (where both sexes jointly respond to rival intruders) and mirror image simulation (where females are isolated from their mate). Plumage manipulation treatments had no measurable impact on female aggression during mirror image simulation tests, though aggression did decrease over the course of multiple assays. Similarly, using simulated territorial intrusion assays, we found no difference in female aggression with respect to both natural coloration and manipulation treatment. Finally, our correlative analysis of natural feather color also revealed no detectable relationship. These findings suggest that female white scapular coloration is unlikely to function as a signal of competitive ability in this tropical species with derived female ornamentation and we discuss possible alternative explanations.

Significance statement

Like their showy male counterparts, female animals may use plumage-based signals to mediate aggressive encounters. Determining if and how signals used in female-female competition may mitigate the costs of agonistic encounters is particularly important, as it is thought to be a key driver of female signal evolution. We used a repeated-measures design and two distinct behavioral assays to explore how both manipulated and natural feather coloration relate to aggression in female white-shouldered fairywrens of Papua New Guinea – a species with female, but not male, plumage polymorphisms. We were unable to detect any differences in aggression with respect to female plumage coloration despite two distinct behavioral assays that mimic distinct social contexts, suggesting that female plumage coloration may function in other contexts or lack a modern signaling function altogether in this system.

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Introduction

Competition over access to limited resources is ubiquitous throughout taxa. When the stakes are high, the resulting conflicts between competitors may impose substantial costs, including injury or even death (Maynard Smith and Price 1973; Parker 1974; Maynard Smith and Harper 1988; Kelly and Godin 2001). Animals may circumvent these costs via the expression of visual signals that reliably indicate social status or quality (Maynard Smith and Price 1973; Johnstone

and Norris 1993; Senar 2006; Santos et al. 2011; Mason and Bowie 2020). The evolution and adaptive function of these "badges of status" are well known for males (Rohwer 1982; Maynard Smith and Harper 1988; Johnstone and Norris 1993), but less is known about signaling dynamics in females. Female signals were classically viewed as a neutral byproduct of sexual selection acting on males (Lande 1980); in this scenario, any investment into ornament production would take away from available resources required for reproduction (Fitzpatrick et al. 1995). However, even among sexually dichromatic species, females are seldom devoid entirely of ornamentation (e.g., Jawor et al. 2004; Leitão et al. 2019a, b; Doutrelant et al. 2020), suggesting that ornament expression may provide socioecological benefits that outweigh any potential physiological costs of expressing them (Doutrelant et al. 2020; Diamant et al. 2021). Moreover, the hypothesis that ornaments are required to be physiological costly to produce (and thus subjected to allocation trade-offs) to honestly signal condition is debated (Biernaskie et al. 2014; Weaver et al. 2017; Doutrelant et al. 2020). Thus, several lines of evidence support that female signals may be under direct selection that is independent of the selection of males (Tobias et al. 2012; Odom et al. 2014; Dale et al. 2015; Wilkins et al. 2020).

There is broad support for the hypothesis that social selection plays an outsized role in the evolution of female ornaments. That is, female ornaments may evolve in response to competition over mate access (i.e., traditional sexual selection) or ecological resources (West-Eberhard 1979, 1983; LeBas 2006; Rosvall 2011; Stockley and Bro-Jørgensen 2011; Tobias et al. 2012; Cain and Rosvall 2014; Doutrelant et al. 2020; Diamant et al. 2021). Although selection for crypsis is often strong in females (Dale et al. 2015; Medina et al. 2017), female ornaments may nevertheless arise due to female-female competition for resources (LeBas 2006), high female reproductive skew (Rubenstein and Lovette 2009), or when sexual selection acts directly on females via male choice (but see Fitzpatrick and Servedio 2017). If the expression of female ornamentation evolves in response to greater competitive demands on females to secure high-quality resources, a relationship between increased ornamentation and increased aggression may be expected (e.g., Murphy et al. 2009; Midamegbe et al. 2011; López-Idiáquez et al. 2016; Leitão et al. 2019a, b). For example, ornamented female tree swallows (*Tachycineta bicolor*) vigorously compete with one another for access to limited nest cavities, and thus, it is not uncommon for physical aggression to be higher in females than in males in this system (e.g., Lipshutz and Rosvall 2021).

When female signals mediate outcomes of intrasexual contests, among-individual variation in signal quality should reflect individual competitive ability. For example, female tree swallows with naturally brighter white body coloration perceive younger, duller females as less of a potential threat (Beck and Hopkins 2019). However, female swallows with experimentally enhanced feather coloration were less able to retain nesting cavities and had lower overall reproductive success than females with experimentally diminished coloration, suggesting social costs may play a role in maintaining signal honesty (Berzins and Dawson 2018; see also Taff et al. 2021). Behavioral differences observed before and after plumage manipulation may be the result of the social environment (i.e., their mates and/or neighboring conspecifics) responding to the altered plumage state (Berzins and Dawson 2018). Therefore, exploring both natural and experimentally manipulated plumage is useful for investigating the potential signaling function of female ornaments. When coupled with multiple behavioral assay types, this approach may yield a more complete picture of female signal function.

Here, we use both an experimental and correlative approach in a species with derived female ornamentation to test if female coloration has a contemporary function in aggressive contexts. We studied white-shouldered fairyw-rens (*Malurus alboscapulatus*), a tropical songbird endemic to New Guinea. Male plumage coloration in this species is a consistent iridescent black-and-white year-round throughout the island (Rowley and Russell 1997; Enbody et al. 2019). However, there are three distinct female polymorphisms across six geographically isolated subspecies, ranging from a drab brown to nearly identical to that of males (Rowley and Russell 1997; Supplementary Fig. S1). Boersma et al. (2020) experimentally induced white scapular (i.e., shoulder feathers) ornamentation in the naturally drab female subspecies (i.e., unornamented female coloration; *M. a. lorentzi*) using testosterone implants (Fig. 1A in Boersma et al. (2020)). In that study, the presence of the white shoulder patch, not exogenous testosterone per se, was associated with increased territorial behavior (Boersma et al. 2020). Although, on the surface, this may suggest that white scapular ornamentation is a possible signal in female-female competitive contexts, the presence of a white scapular patch is not a naturally occurring phenotype for this subspecies. Thus, it remains unclear whether this plumage patch is a signal used in intra-sexual contests in naturally ornamented subspecies. As increased social selection pressures appear to be associated with the evolution of female ornamentation in this species (Karubian 2013; Enbody et al. 2018; Jones et al. 2021) and the emergence of black-and-white coloration in this subspecies is a derived trait (Driskell et al. 2011; Johnson et al. 2013; Karubian 2013) that likely arose in response to direct selection, rather than drift (E.D. Enbody et al., unpubl. data), this remains an important question to resolve.

We coupled a plumage-based manipulation with two behavioral assay techniques to experimentally test for a relationship between female plumage ornamentation and

Fig. 1 Experimental design of the current study on white-shouldered fairywrens of Papua New Guinea. (**A**) Our order of operations. First, we conduct a simulated territorial intrusion (STI). Second, we captured individuals and females received their first mirror image stimulation (MIS) trial > 15 min first capture. We then applied one of three possible treatments to females, followed immediately by a second mirror image stimulation assay. We repeated this process seven days post-manipulation. (**B**) Females post plumage manipulation: (left to right) control, minor manipulation, and major manipulation treatments. (**C**) Diagrammed experimental cage setup. The cage was set up with three perches of equal height spaced evenly throughout. All-but-one sides were covered in a white cloth to reduce external stimuli and reduce perceived possible exits for the focal bird. The mirror was coved by a removable (from a distance) cover, after which the bird was exposed to its mirrored image for 7 min. We analyzed the proportion of time spent in each zone relative to the mirror (close, neutral, or far from the mirror). Fairywren cartoon reproduced and edited (**A**, **B**) from a silhouette available copyright free from "Creazilla" (https://creazilla.com/) or (**A**, **C**) with permission from Allison Johnson. Fairywren photo credits: John Anthony Jones
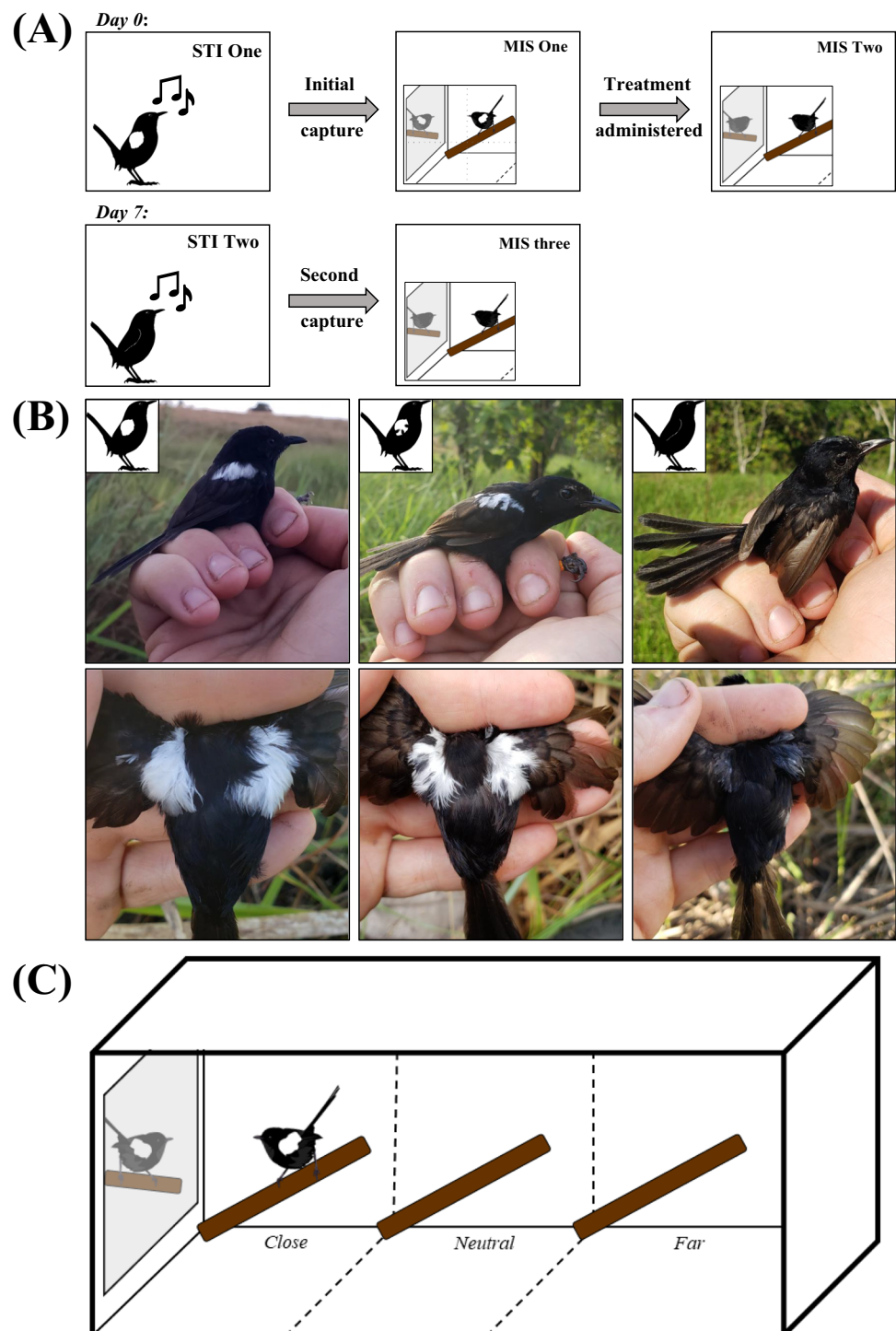
territorial aggression. We additionally used a correlative approach to explore if natural feather coloration (measured via reflectance spectroscopy) and ornament size correlate with territorial aggression during simulated territorial intrusions. We assessed aggressive behavior via (1) mirror image stimulation assays that pitted individuals against their own mirrored reflection (Gallup 1968; Leitão et al. 2019a) as well as (2) a pair of simulated territorial intrusions that provide

another context for assessing relationships between plumage and aggression while also allowing us to test for differences in aggression in response to any changes in the social environment. In particular, because male and female fairywrens respond to territory threats as a coordinated duo (Enbody et al. 2018; Jones et al. 2021), our experimental design allows us to assess if male behavior changes in response to their mate's manipulated plumage. We predicted that, in

female white-shouldered fairywrens, feather coloration and patch size are positively correlated with the degree of territorial aggression. Moreover, we predicted that if female scapular coloration is a signal used in competitive contexts, individuals would be less aggressive to reflections of manipulated birds that are perceived as less ornamented and thus less of a threat (sensu Beck and Hopkins 2019; Leitão et al. 2019a, b). Support for these predictions would suggest that female coloration serves as a signal in competitive contexts. Finally, we predicted female territorial aggression will decrease over time in individuals with experimentally diminished ornaments relative to our control treatment, consistent with changes in the social environment (unmeasured in this study) playing a role in shaping an aggressive response.

Methods

Study species and general field methods

White-shouldered fairywrens (*M. alboscapulatus*) are an insectivorous grassland species commonly seen in community gardens and savannahs throughout New Guinea (Rowley and Russell 1997; Enbody et al. 2019). As is the case in many New Guinean species, research is limited on our focal fairywren relative to the more widely studied Australian species (Rowley and Russell 1997). White-shouldered fairywrens exhibit year-round territoriality and breeding (Enbody et al. 2019); while this differs from closely related congener species (Rowley and Russell 1997), this is common in many tropical species (Stutchbury and Morton 2001), including Australian fairywrens near the equator (Hall and Peters 2008; Leitão et al. 2019b). As in other fairywren species, *M. a. moretoni* (the focal subspecies of the current study and where females are ornamented) occasionally have helpers at the nest, suggesting cooperative breeding (anecdotally, helpers may be both sexes, Enbody et al. 2019). Relative to an unornamented subspecies of white-shouldered fairywrens (*M. a. lorentzi*), territories tend to be smaller and both sexes are more aggressive in their territory defense than their unornamented counterparts, likely due to increased social selection pressure (Enbody et al. 2018, 2019; Jones et al. 2021). Finally, female *M. a. moretoni* have shorter tails than *M. a. lorentzi* (Enbody et al. 2019; Jones et al. 2021); shorter tails are thought to serve as a signal of social dominance (Karubian et al. 2009), and several *Malurus* fairywren species exhibit this pattern (Swaddle et al. 2000).

In the current study, we studied a population of *M. a. moretoni* in Podagha Village (9.692°S, 149.895°E), Milne Bay Province, Papua New Guinea, from June to July 2019, at the beginning of the dry season. It was not possible to record data blind because our study involved focal animals in the field. In general, our order of operations was to (1) perform a simulated territorial intrusion on birds, (2) capture those individuals via mist-nets for banding and standard morphometric measurements (*correlative analysis*, below), (3) expose these individuals to a mirror image simulation assay after being held for > 15 min (i.e., the approximate time that circulating corticosterone has reached its asymptotic peak, thus not influencing the behaviors observed during mirror assays, Cockrem and Silverin 2002). Next, we (4) applied one of three possible plumage manipulation treatments to females and (5) immediately conducted a second mirror assay. We repeated steps 1–3 seven days post-treatment to complete a before-and-after assessment of the effects of plumage manipulation (Fig. 1A).

Because this was a previously unbanded population, the exact ages of individuals tested are unknown. We estimated age via skull ossification and only manipulated the plumage of paired adults after observation during simulated territorial intrusion assays. This species has the capacity to breed year-round and breed asynchronously (Enbody et al. 2019), and thus, we included both breeding and non-breeding individuals in this study. We included breeding status (estimated as either "breeding" or "non-breeding" by the presence/absence of an active brood patch or juveniles on territory) as a fixed effect in our analyses.

For our species, there is good justification for focusing on the white scapular patch of female fairywrens. This feather patch was induced via exogenous testosterone implantation in a naturally occurring unornamented subspecies (*M. a. lorentzi*; a subspecies where the white patch naturally is absent in females). Expression of the white scapular enhanced the territorial singing behavior of the manipulated female, although it did not alter the behavior of the mated male nor did it appear to impose a social cost to ornament expression (Boersma et al. 2020). However, and importantly, the presence of white scapular per se, and not exogenous testosterone, was responsible for behavioral differences observed. Indeed, testosterone did not elevate aggression prior to the development of the white shoulder, and concentrations were not elevated at the time of behavioral analysis in that study (Boersma et al. 2020). Females within subspecies all resemble one another; thus, every female in the current study (*M. a. moretoni*) is fully black and white alongside males. Nonetheless, they remain dimorphic, as males are iridescent throughout their body and females are matte black and have brown wings feathers (Enbody et al. 2019).

Experimental approach

Plumage manipulation

Adult females were assigned to one of three possible treatment groups in a randomly determined order: "major" manipulation, "minor" manipulation, and control (*n* = 15

individuals per treatment; Fig. 1B). In the two experimental treatments, we reduced the size of the scapular patch by coloring white feathers black using an alcohol-based marker (ChartPak™: "Super Black"); we chose alcohol-based markers for the manipulation assays to ensure that the plumage would remain altered for at least one week in a humid environment. For the major manipulation treatment, we colored feathers entirely black to remove the putative signal altogether, whereas, for the minor manipulation treatment, we colored only along the edges of the scapular feather patch in three small (~2–3 mm in length) locations to reduce the size and immaculateness of the putative ornament (Ferns and Hinsley 2004). For some individuals in the heavy treatment, the white scapular itself was more "peppered" than entirely black seven days post-manipulation. These plumage patches were nonetheless significantly dampened compared to the minor and natural plumage colors, and no individual of either manipulation treatment had a plumage patch that appeared "normal" after seven days. As a control, we covered the shoulder patch using a clear, non-toxic water-based marker (Ecoline™: "Blender") that did not alter the color of the feathers when tested under a spectrometer prior to the field season.

Both plumage manipulations are beyond what we observe in the wild naturally. Although there are some variations in the evenness of the ornament itself, these are rare and usually consist of a minor brown patch along the edge of the scapular patch (more commonly seen in juveniles and rarely in adults). Our minor manipulation made three small indentions into the putative ornament was an attempt to mirror this but enhance its efficacy. On the other hand, our major manipulation is not a phenotype that is observed in the wild. Every adult female seen has a full scapular patch, and the only stage in their life history that lacks it is immediately after leaving the nest. We did not "enhance" the shoulder patch by enlarging the white putative ornament as the only way to reliably do this in melanin-based black feathers involves bleaching the feathers, and we opted not to do this both for ethical considerations as well as we did not want the chemical treatment to disproportionately affect the outcome beyond the scope of the study. Moreover, we were unsure if patch size was itself a signal, and thus, we measured patch size in this study as a first step to answer this question. We tested the markers used in this study with a reflectance spectrometer prior to the field season and found them to be as close as possible to the natural black color found on free-flying fairywrens (mean marker brightness: 5%; mean natural brightness: ~2–3% (Enbody et al. 2019)).

Mirror image stimulation

Assays occurred between 0600 and 1100 or between 1530 and 1730 local time (GMT + 10) while avoiding intense heat to mitigate any additional unnecessary stress to the focal individual. We interpret any aggressive response as a response to a perceived same-sex rival (sensu Leitão et al. 2019a). Following a similar methodology as Leitão et al. (2019a), we temporarily placed fairywrens inside a 60 cm (length) × 40 cm (width) × 40 cm (height) cage located within their own territory and covered by a white cloth on all but one side (Fig. 1C). The cage had three perches at identical heights but at different distances from the mirror (categorized as close, neutral, and far). We allowed for 5 min of acclimation to the cage before exposing females to their mirrored reflection for 7 min. We continuously measured the proportion of time spent at each distance class and enumerated each act of (presumed) aggressive behavior (i.e., physically striking the mirror, threatening displays/poses, and soft songs (Supplementary Fig. S2)). Soft songs are thought to be a reliable indicator of aggressive intent (e.g., Templeton et al. 2012), but we do not currently know if this is the case in white-shouldered fairywrens per se. Nevertheless, no assayed individual sang a song at full, or even near full, volume during the mirror trial, and any songs that were performed were infrequent. All behaviors and the time spent within each distance class were quantified separately for the acclimation period and mirror reflection period. Aggressive behaviors were only seen when the mirror was exposed; during the acclimation period, the majority of time was spent attempting to find an exit to the cage (pers. obs.). We video-recorded each trial with a partially camouflaged GoPro Hero CHDHA-301 (San Mateo, CA) or Sony HDR-CX405 Handycam (Tokyo, Japan).

We exposed each focal individual female to three separate mirror stimulation assays. In the first assay (≥ 15 min post first capture; mean time elapsed: 16.5 min), females were exposed to their mirrored reflection without a plumage manipulation to determine a baseline aggression profile and to identify any correlates with aggression (e.g., body size and scapular size). Immediately following the first trial, female ornaments were manipulated, and individuals were subjected to a second assay. Immediate testing after plumage manipulation precludes external social interactions from influencing manipulated female's behavior, thus providing a baseline response to how individuals perceive diminished ornaments (i.e., individuals of presumed "lower quality") to directly compare to their first aggression profile. Finally, we recaptured females and re-tested their response to their mirrored reflection one-week post-manipulation to characterize any behavioral differences over time that may reflect if and how the social environment influences their response to scapular ornamentation. Changes in an individual's behavioral responses between captures may indicate a feedback loop between the focal individual and its social environment, such that changes in how neighbors perceive and respond to the

focal bird in response to manipulation in turn may influence her own response to others.

Simulated territorial intrusions

We conducted two simulated territorial intrusion trials for each individual: prior to initial capture (i.e., pre-manipulation) and prior to their second capture (seven days post-manipulation; Fig. 1A. We broadcasted a randomly chosen *M. a. moretoni* female song exemplar ($n=5$) and along with a female cardstock mount ($n=4$) for 5 min total, followed by two 2 min silence (Jones et al. 2021). Our cardstock mounts were adapted from a generic wren design available by the artist Johan Scherft (http://www.johanscherft.com) as used in Enbody et al. (2018) and Jones et al. (2021). Briefly, each mount was painted to resemble the female phenotype, and collected tail feathers were attached in an upright position. We noted the following behaviors as they occurred during the trial: mean distance (in m) from the mount, the proportion of time spent <5 m of the mount, attack rate (aggregated sum of the number of flybys (within 2 m of the mount) and dives (<0.5 m from the mount)), and song rate (both duets and individual songs). We recorded our song exemplars from a geographically distinct population of fairywrens during previous field seasons using a Marantz PMD 661 Mk II (96 kHz sampling rate, 24-bit depth, D&M Professional, Itasca, IL) and a Sennheiser ME66 shotgun microphone and K6 power module (Sennheiser Electronic Corporation, Old Lyme, CT). We edited playbacks in Audacity v. 2.2.2 to filter out noise below 500 Hz and standardize amplitude (Audacity Team 2018). Each playback is a single female song repeated every 10 s for the length of the trial and was broadcasted using a Samsung (Suwon-si, South Korea) Galaxy S9 over Bluetooth via an Ultimate Ears Roll 2 speaker (Irvine, CA, USA).

Correlative approach

Morphological analysis

Alongside the experimental manipulation, we sought to explore the correlative relationship between aggression and aspects of body size, patch size, and the color of unmanipulated ornaments. Upon the capture of individuals involved in the current study, we recorded tail length (a putative signal of social dominance; Karubian et al. 2009), tarsus length (± 0.01 mm), and mass (± 0.01 mg). We used standardized residuals of regression between tarsus length and mass to get an estimate of body condition. Finally, we took standardized extended-wing and profile photographs (on the first capture, pre-manipulation) of each focal individual. From these, we calculated the area of the scapular patch (mean $\pm$ SD: 212.59 ± 42.25 mm^2) and the length of the

eyebrow (2.72 ± 1.59 mm; a plumage patch with considerable among-individual variation among females but is absent in males) using image analysis tools in ImageJ (Schneider et al. 2012).

Plumage color analysis

We decided against pulling feathers from individuals in the focal population of the current study because feather removal could influence perceptions of signal size and color beyond that intentionally produced by plumage manipulation. As such, we assessed the relationship between individual coloration and territorial aggression using feathers and aggression data sampled in a separate behavioral study (Jones et al. 2021). Using a similar simulated territorial intrusion methodology as the current study (i.e., the same behavioral responses to simulated stimuli), the authors tested female aggression in response to the various song and plumage exemplars. Here, we correlate those aggressive responses to feather coloration (using feathers collected at the time of the study) via reflectance spectrometry.

We made three repeated measures of reflectance for crown (black) and scapular (white) regions using a USB2000 + spectrometer (R400-7-UV–VIS probe, RPH-1 probe holder) with a PX-2 pulsed xenon light source in OceanView software (Ocean Optics, Dundin, FL, USA). Reflectance spectra generated (in 5 nm steps) were calibrated relative to a white standard that reflects 100% of light evenly from 300 to 700 nm (Ocean Optics WS-2). We chose crown feathers over other black regions because female crown coloration exhibits more variation than the other melanin-based black plumage patches in this species and signaling color patches are expected to be more variable than non-signaling patches (Delhey and Peters 2008). Moreover, we suspect that this body region also is likely to contrast with the putative white scapular ornament.

We used a psychophysical model of avian vision (Vorobyev and Osorio 1998; Vorobyev et al. 1998) to explore plumage variation from the bird's perspective, implemented by Delhey et al. (2015) following the formulas in Cassey et al. (2008). Importantly, we found that using the formulas presented in Montgomerie (2006) yielded identical biological results. Avian color vision is thought to be mediated by four types of single cones present in bird retinas that are sensitive to and detect variation in chromatic coloration (very short (VS), short (S), medium (M), and long (L) wavelengths) as well as one type of double cone that mediates achromatic sensitivity (Cuthill 2006). Avian vision can be further split into whether species are U- or V-type species, with the former being those species that have the highest sensitivity to UV light (although the latter nonetheless maintains the ability to perceive UV light; Delhey et al. 2013; Ödeen and Håstad 2013). As

white-shoulder fairywrens are a V-type species (Ödeen et al. 2012), we used the mean V-type peak avian sensitivity of 416 nm, 478 nm, 542 nm, and 607 nm (Endler and Mielke 2005). Signal-to-noise ratios for each cone type were calculated using formula 10 in Vorobyev et al. (1998) using the mean proportion of cones present in V-type birds from Hart (2001: VS = 0.381, S = 0.688, M = 1.136, L = 1.00), a Weber fraction of 0.1 for the L cone (Olsson et al. 2018). We set our irradiance spectrum to that of standard daylight (D65; Vorobyev et al. 1998).

This approach reduces the measured spectrum to a set of three ("X," "Y," and "Z") chromatic coordinates that define their position in avian tetrahedral colorspace. In this visual space, the X axis represents the relative stimulation of the S cone relative to the VS cone; the Y axis represents the relative stimulation of the M cone to both VS and S cone; the Z axis is the relative stimulation of the L cone to VS, S, and M cones. The resulting chromatic distance values are reported in just noticeable differences (JNDs), where values representing > 1 are considered distinguishable differences.

Following Delhey et al. (2015) and Leitão et al. (2019a), we summarized XYZ coordinates with a separate principal components analysis per plumage region using a covariance matrix. All PCA analyses resulted in one factor (hereafter: $PC1_{chroma}$) that explained > 94% of the variation (Table 1). However, despite explaining an overwhelming amount of the variation found within each plumage patch, there is very little variation overall within both colors and both PCs resulted in < 1 for their eigenvalue (Table 1; see also Supplementary Fig. S3 for graphical representation of this limited variation). Both plumage regions have large, negative values for both the Y and Z axes and load higher on the X axis (with shoulder feathers loadings greater than crown loadings). This indicates that these colors stimulate the VS and S cones higher than the M and L cones (Supplementary Fig. S3). In other words, positive $PC1_{chroma}$ values are colors richer in

shorter wavelengths (UV/blue) than in longer wavelengths (red).

Because chromatic variation among individuals within each feather patch appears to be relatively limited, we also explored achromatic variation (i.e., variation in overall brightness). We estimated the overall brightness of the crown and shoulder by calculating the achromatic contrast between each patch and a reference spectrum that sets a low value for the double cone quantum catch (i.e., close to zero; 0.001), thus simulating an exceptionally dark spectrum. We additionally calculated the contrast (both chromatic and achromatic) between the two plumage patch regions using the *coldist* function available in the R package pavo (v.2.0; Maia et al. 2019). For both achromatic analyses, we used the double cone visual sensitivity of the blue tit (*Cyanistes caeruleus*), as these data for our species are not available. Contrast is measured in JNDs.

Statistical analysis

Mirror image stimulation

We performed all statistical analyses in R v. 4.0.4 (R Core Team 2021). We aggregated mirror strikes, pecks, songs, and displays in response to exposure to one's mirrored reflection into one "total aggression" value, summed as the frequency of the behavior controlled for the length of exposure (sensu Leitão et al. 2019a); while striking and pecking at the mirror are correlated traits (due to being different forms of "attacking" their mirrored reflection), songs and display rates were infrequent and are not significantly correlated with attack rate in either direction. We then identified a composite index of aggressive behavior by running a principal components analysis (PCA) using this aggression score (hereafter Mirror-PC1) as well as the proportion of time spent close to and far away from the mirror using the "prcomp" command from the "stats" package after scaling and centering our data. Mirror-PC1 generated one component with an eigenvalue > 1.0, explaining 63.7% of the variation (Table 2).

We tested the effects of treatment (plumage manipulation) and trial order (i.e., pre-manipulation, immediately post-manipulation, and one week following manipulation) and estimated breeding status (i.e., currently breeding or not) on individual aggression (Mirror-PC1) using a linear mixed-effects model via the R package lme4 (Bates et al. 2015). We included individual ID. We tested this and subsequent linear mixed-effects models to ensure they did not violate the assumptions of normality nor heteroscedasticity. Our response variable (Mirror-PC1) failed to meet the assumptions of normality as it had a right-skewed bell-shaped curve; the removal of outliers that skewed the curve resulted in a normal distribution, but whether these outliers

Table 1 Loadings for principal components analysis of *XYZ* coloration ($PC1_{chroma}$) for white-shouldered fairywrens

	Shoulder patch	Crown
Eigenvalue	0.02	0.73
Proportion of variance	0.94	0.98
X	0.555	0.244
Y	− 0.633	− 0.58
Z	− 0.54	− 0.777

Loadings are for a single component for each feather patch which explain the majority of variation across patches. The X axis represents the relative stimulation of the S and VS cones relative to M and L, with positive values corresponding with greater stimulation of the VS cone relative to the S cone; the Y axis is the relative stimulation of the M cone to both VS and S cone; the Z axis is the relative stimulation of the L cone to VS, S, and M cones

Table 2 Loading scores for the principal component analysis exploring how individuals respond to mirror image stimulation after exposure to the mirror (left, Mirror-PC) and free-flying individuals responded to simulated territorial intrusions (right, STI-PC)

	Mirror-PC1	STI-PC1	STI-PC2
Eigenvalue	1.852	2.271	1.219
Proportion of variance	0.618	0.454	0.244
Aggression (strikes + pecks + displays + songs)	0.500		
Proportion of time spent close	0.650		
Proportion of time spent far	− 0.573		
Latency to respond to playback		− 0.225	− 0.628
Attack rate		0.410	0.005
Proportion of time spent < 5 m		0.620	− 0.172
Average distance		− 0.630	0.049
Song rate		− 0.008	0.757

remained or not in our main dataset did not impact the biological interpretation of our results, and thus, we opted to retain them.

Simulated intrusion response

We ran a second PCA to test whether individuals change their responses to simulated territorial intrusions before and after manipulation using the behavioral responses observed during these assays (defined above). The top two components of this PCA (hereafter: STI-PC1 and STI-PC2) together accounted for 69.8% of the variation (Table 1).

We assessed the effects of plumage treatment, simulated territorial intrusion order (pre- or post-manipulation), sex, and breeding status on both STI-PCs using a linear mixed-effects model. We included individual ID (nested within group ID), whether birds were located via a playback as random effects along with our mount ($n = 4$) and song exemplars ($n = 5$). Both response variables (STI-PCs) follow a normal distribution.

Correlational analysis

We explored if natural feather coloration and body size measurements were associated with aggression via a series of correlative analyses in the R package "psyche" (Revelle 2021). First, for individuals who we experimentally manipulated, we explored if tail length, body condition (i.e., mass controlled for tarsus length), scapular patch size, and eyebrow length correlated with aggression data (all morphology values were taken on the first capture and thus pre-manipulation). Second, using data taken from Jones et al. (2021; a separate behavioral study), we tested for multiple correlations between components of territorial aggression (i.e., the

PCs from Jones et al. 2021) and plumage coloration from feathers taken during that study. As these data were non-normally distributed, we ran Spearman's correlation matrix using Holm's correction for multiple correlations. Finally, we collected blood samples for testosterone analysis (sensu Enbody et al. 2018), but our resulting sample size was prohibitively low; we did not find any significant relationship involving testosterone and behavior (reported in Supplementary information).

Results

Experimental results

Mirror image stimulation response

There was considerable variation in the frequency in which females struck (mean ± SD: 10 ± 10.15 attacks min^{-1} (range: 0–42.65)) and pecked at the mirror (0.15 ± 0.42 pecks min^{-1} (range: 0–2.54)) as well as displayed (0.03 ± 0.25 displays min^{-1} (range: 0–2.61)) and sang at their mirrored reflection (0.13 ± 0.57 songs min^{-1} (range: 0–0.45)). Most individuals aggressively postured themselves directly in front of the mirror throughout the duration of the trial (i.e., a greater proportion of time spent near the mirror: 0.71 ± 0.23), although some kept their distance (proportion of time spent far away from the mirror: 0.06 ± 0.08).

We interpret higher loading scores of Mirror-PC1 as individuals who responded most aggressively (Table 2) by spending a greater proportion of time near the mirror and attacking it more frequently. We exposed 45 females to at least one mirror image stimulation each, but ended up with 13 control females, 15 minor manipulations, and 11 major manipulations with all three mirror assays performed (Supplementary Table S1 for the breakdown of sample sizes per treatment per trial order). We first tested if there was an interaction between trial order and treatment; upon finding that this was not statistically significant ($p = 0.35$), we removed the interaction term and re-ran the model. Treatment did not predict the aggressive response ($p = 0.17$), but we found that individuals became less aggressive overall after each subsequent mirror assay ($p < 0.001$; Table 3, Fig. 2).

Simulated intrusion response

We interpreted higher loading scores for STI-PC1 as individuals who spend a greater proportion of time near the mount and attack it more frequently, whereas STI-PC2 is associated with birds that respond quicker to playback and counter-sing more often. We assessed how pairs respond to simulated territory intrusions both before and after plumage manipulation

Table 3 Summary statistics for the effect of plumage manipulation on aggressive behaviors seen during mirror image stimulation (MIS) and simulated territorial intrusions (STI)

Response variable	Fixed effect	Estimate	SE	Sum of squares	F	df	p
Mirror-PC1	Treatment	−0.588	0.40	2.680	1.903	2, 42.77	0.162
	MIS order	0.126	0.39	27.023	19.194	2, 82.51	**< 0.001**
	Breeding status	−1.117	0.19	0.104	0.148	1, 42.76	0.702
	Random effect	*Variance*					
	Individual ID	0.86					
	Residuals	0.70					
STI-PC1	Treatment	0.153	0.23	2.547	1.206	2, 46.190	0.309
	STI order	−0.298	0.22	110.437	104.59	1, 133.19	**< 0.001**
	Sex	−1.153	0.11	0.026	0.025	1, 128.65	0.875
	Breeding status	−0.033	0.16	0.576	0.546	1, 46.64	0.464
	Treatment*STI order	0.251	0.34	6.629	3.139	2, 133.40	**0.047**
	Random effect	*Variance*					
	Individual ID/group ID	<0.001					
	Group ID	0.44					
	Prior song heard? (Y/N)	<0.001					
	Residuals	1.06					
STI-PC2	Treatment	−0.236	0.20	0.838	0.866	2, 45.92	0.427
	STI order	0.117	0.20	34.704	71.754	1, 131.15	**< 0.001**
	Sex	0.646	0.08	2.402	4.967	1, 128.06	**0.028**
	Breeding status	0.236	0.11	0.857	1.771	1, 46.22	0.190
	Random effect	*Variance*					
	Individual ID / Group ID	<0.001					
	Group ID	0.45					
	Prior song heard? (Y/N)	<0.001					
	Residuals	0.48					

Treatment refers to the three plumage manipulation treatments, "MIS order" refers to the three mirror image stimulation assays (before manipulation, immediately after, and one-week post-manipulation), and "STI order" refers to STI's pre- and post-manipulation. Statistically significant relationships are presented in bold type

Fig. 2 Effect of plumage manipulation on aggressive behaviors during mirror image stimulation (MIS). There is no effect of treatment on response, although the intensity of aggression observed decreased with repeated. Boxes represent the interquartile range (IQR), with the median depicted as the central line. Whiskers extend to 1.5*IQR, and points beyond this are outliers
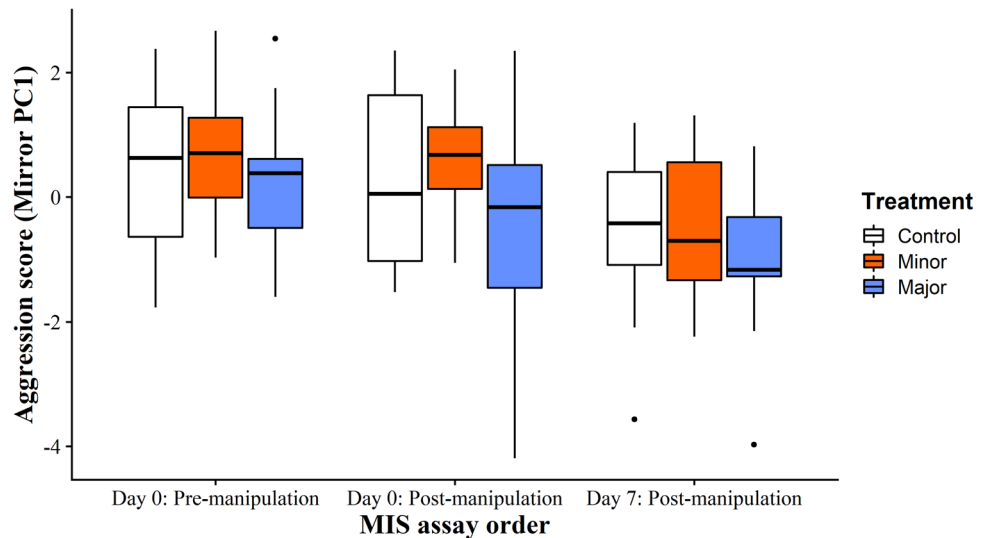
for 15 control groups, 14 minor manipulations, and 12 major manipulations (Supplementary Table S1).
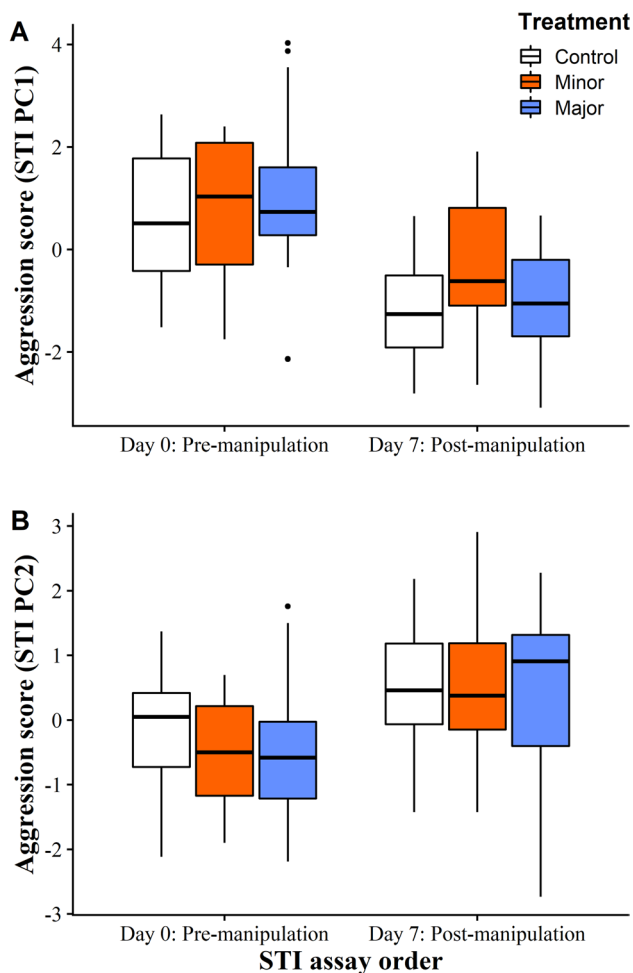

Fig. 3 Effect of plumage manipulation on aggressive behaviors observed during simulated territorial intrusions (STI) before ("Day 0") and after ("Day 7") manipulation. There is no effect of treatment on response, but individual behavior varied between repeated assays. We found that (**A**) overall aggression (STI-PC1) decreased (i.e., fewer attacks and individuals were further from the playback exemplar), but (**B**) overall attentiveness (STI-PC2) increased (i.e., individuals responded quicker and counter-sang more frequently) with repeated assays. Boxes represent the interquartile range (IQR), with the median depicted as the central line. Whiskers extend to 1.5*IQR, and points beyond this are outliers

We found no significant interaction for either STI-PCs with sex and thus removed this interaction term from each model. However, we found a significant interaction between treatment (i.e., plumage manipulation) and assay period (pre- or post-manipulation) on STI-PC1 ($p = 0.05$; Supplementary Fig. S4). Aggression scores on the STI-PC1 axis decreased after manipulation for all treatments ($p < 0.001$; Table 2, Fig. 3A), but STI-PC1 scores declined less among individuals who received a minor plumage manipulation than those who received either a control or major manipulation treatment. We found no significant main effect of treatment ($p = 0.26$) nor sex on STI-PC1 ($p = 0.88$).

We did not find any statistically significant interaction involving plumage manipulation treatment and assay period on STI-PC2. Thus, all interaction terms were removed, and models were re-run. Again, we found no statistical difference in STI-PC2 among treatments ($p = 0.54$; Table 3, Fig. 3B), but individual STI-PC2 scores did increase between pre- and post-manipulation ($p < 0.001$). Thus, birds responded more quickly and counter-sang more often in post-manipulation intrusion assays than during pre-manipulation, regardless of which treatment they received. There was also a difference between the sexes, such that male STI-PC2 scores were higher than those of females ($p = 0.04$; Table 3).

Correlational analysis

Using aggression data generated from both mirror assays and simulated intrusion assays along with body morphology data from individuals prior to plumage manipulation (i.e., Mirror-PC1, STI-PC1, and STI-PC2), we did not find a statistically significant relationship between any condition or status index and aggression (all $p > 0.20$; Table 4).

To explore correlations between aggression and coloration, we reproduced a PCA from Jones et al. (2021) and compared aggressive responses to simulated territorial intrusion assays to the coloration of feathers taken from those individuals (see Table 1 in Jones et al. (2021) for PC loadings). There, the authors used a reciprocal playback experiment that varied the signal exemplar stimuli that focal individuals experienced (ranging from a subspecies that did, or did not, express female plumage ornamentation). Briefly, higher values along this PC axis tend to correspond with overall more aggressive individuals; (1) PC1 is associated

Table 4 Correlation between multiple body condition indices and responses to both mirror image stimulation (Mirror-PC1) and simulated territorial intrusion (STI-PC1, STI-PC2)

Condition index	Mirror-PC1			STI-PC1			STI-PC2		
	r_s	n	p	r_s	n	p	r_s	n	p
Mass (adj. for tarsus length)	0.013	42	0.93	−0.09	41	0.56	−0.08	41	0.64
Tail length (mm)	0.10	41	0.55	−0.01	38	0.97	0.11	38	0.50
Scapular patch area (mm²)	−0.02	43	0.89	0.21	40	0.20	0.09	40	0.57
Eyebrow length (mm)	0.03	41	0.85	0.12	36	0.48	−0.12	36	0.48

with individuals that respond quicker to stimuli, spend more time near the mount, and frequently fly-by that mount, (2) PC2 is associated with individuals that those sing songs alone rather than as a duet and also attack more frequently, and (3) PC3 is associated with individuals those that tend to respond quickly and sing more frequently in response to stimuli, but keep a distance of > 5 m on average. In the current study, we used the behavioral responses that focal individuals made when the exemplars experienced matched their local subspecies for both song and color phenotype. In doing so, we assure that our correlation analysis is testing individual behaviors in response to similar and ecologically relevant stimuli. Nevertheless, we were unable to find a significant relationship between female coloration and individual behavior after applying Holm's correction to account for multiple correlations (all $n = 18$, $p > 0.99$; Table 5).

Discussion

We investigated whether the white scapular patch of female white-shouldered fairywrens is a signal of competitive ability by pairing an experimental manipulation of plumage coloration with two distinct behavioral assays that simulate alternative social contexts alongside a correlative analysis of plumage coloration for unmanipulated feathers. Results from both mirror image stimulation (experimental) and simulated territorial intrusions (both experimental and correlative) suggest that scapular coloration in females is unlikely to be used as a signal during female-female aggressive interactions in *M. a. moretoni*. Female aggression did not vary in response to plumage manipulation in either of the assays we conducted. Moreover, there was no evidence that either males or neighboring pairs appear to perceive diminished scapular ornamentation as a reduction in status, as inferred by the absence of any treatment-specific change in individual behavior in both simulated territorial intrusions and mirror assays one-week post-manipulation.

These results contradicted our a priori hypotheses because black-and-white coloration in females is evolutionarily derived from the subspecies we studied (Driskell et al. 2011; Johnson et al. 2013; Karubian 2013; E.D. Enbody unpubl. data) and prior work suggests that female ornamentation may have evolved in response to increased social selection pressure (Jones et al. 2021). Although social selection is an often-cited mechanism promoting female ornamentation, with particular emphasis on competition over ecological resources (West-Eberhard 1979, 1983; LeBas 2006; Rosvall 2011; Stockley and Bro-Jørgensen 2011; Tobias et al. 2012; Cain and Rosvall 2014; Doutrelant et al. 2020; Diamant et al. 2021), our results point to alternative explanations for the contemporary function of female white-shouldered fairywren coloration.

One possible explanation is that plumage coloration in female white-shouldered fairywrens serves a contemporary signaling function in other, non-aggressive contexts. For example, male mate choice associated with sexually selected signals in females has been reported in a variety of taxa and includes species where ornamentation is either mutual or is female-specific (reviewed in Nordeide et al. 2013; Fitzpatrick and Servedio 2018). These scenarios typically occur when female traits are correlated with heightened fecundity or otherwise provide a direct benefit for male choosiness (Bonduriansky 2001; LeBas et al. 2003; Griggio et al. 2005; Cole and Endler 2016; Courtiol et al. 2016; Fitzpatrick and Servedio 2018). In the tropics, longer lifespans coupled with year-round territoriality and partnerships probably result in fewer vacant territories and potential partners and thus higher levels of competition for breeding opportunities for both sexes (Slater and Mann 2004). Greater selection may thus act on males to prefer higher-quality females, consequently leading to a role for signals that accurately reflect female quality and condition (Stutchbury and Morton 2008; Freeman et al. 2020; but see Hegyi et al. 2015). However, that male behavior did not change in response to plumage treatment (although they also became less aggressive with

Table 5 Spearman's rank-sum correlation between female plumage coloration and the top three components explaining individual behavioral response to simulated territorial intrusions (via the behavioral principal components analysis (PCA) reproduced from Jones et al. (2021))

	PC1			PC2			PC3		
	r_s	p	adj. p	r_s	p	adj. p	r_s	p	adj. p
Chromatic variation									
Crown-PC1	−0.25	0.32	0.99	−0.08	0.75	0.99	0.56	0.02	0.99
Shoulder-PC1	−0.31	0.21	0.99	−0.18	0.47	0.99	0.20	0.44	0.99
Contrast between regions	0.12	0.63	0.99	0.08	0.76	0.99	−0.23	0.36	0.99
Achromatic variation									
Crown brightness	0.47	0.05	0.99	0.08	0.75	0.99	−0.45	0.06	0.99
Shoulder brightness	0.15	0.55	0.99	0.10	0.69	0.99	0.28	0.26	0.99
Contrast between regions	0.24	0.33	0.99	−0.07	0.79	0.99	0.48	0.05	0.99

All correlations are pairwise ($n = 18$). P values are listed for the uncorrected analysis alongside adjusted P values using Holm's correction for multiple correlations

repeated assays) may stand in contrast to this explanation. Males remained as aggressive during simulated territorial intrusions as females, suggesting that their relative investment in the maintenance of the pair bond remained consistent and not responsive to female plumage change. With that said, Chaine et al. (2018) found plumage manipulation only fools strangers and not individuals familiar with the manipulated individual. This may explain, at least in part, why male behavior did not vary over the course of the study.

Alternatively, it is plausible that female plumage ornamentation in this species does not serve a contemporary function. Indeed, the current expression of a trait is not necessarily indicative of the conditions and selection pressures that occurred when the trait evolved (Gould and Vrba 1982; Autumn et al. 2002; Cardoso and Mota 2010; Kraaijeveld 2014), and ornamental traits exhibit considerable evolutionary lability in female animals (Wiens 2001; Omland and Hofmann 2006; Hofmann et al. 2008; Cardoso and Mota 2010; Kraaijeveld 2014). It is also possible that female ornamentation arose in this system as a selectively neutral trait due to shared genetic pathways with males (Lande 1980). For example, if heighted competition produced females with elevated baseline testosterone concentrations relative to ancestral populations with unornamented females (Enbody et al. 2018) and elevated androgens induces expression of white scapulars in females (Boersma et al. 2020), then the expression of the white scapular may be a byproduct of selection for elevated androgens. More research, including genomic approaches to detect signatures of selection, are required to fully resolve this hypothesis.

Many species display complex signaling phenotypes, comprised of multiple distinct plumage patches and patterns which may be derived from various physiological mechanisms (e.g., in birds, pigmentation versus structural coloration; Hill and McGraw 2006). Here, we aimed to explore the full plumage phenotype in *M. a. moretoni* by examining the putative non-ornamental black coloration on the crown and calculating the contrast between this feather patch and the scapular. Nevertheless, we were unable to detect any correlative relationships between aggression and natural coloration (of the crown, the shoulder, and the contrast of the two regions) nor in manipulated plumage throughout both behavioral assay types. Females exposed to their mirrored reflection decreased their overall aggression profile (i.e., lower Mirror-PC1 scores) over time, but the behavioral difference between their first mirror trial (where no plumage manipulation was applied) and subsequent mirror trials did not vary among treatments (Fig. 2). Both sexes were less aggressive during simulated territorial intrusions on their second assay (i.e., post-manipulation lower STI-PC1 scores), but this too did not vary among treatments. Interestingly, both sexes were more responsive on their second simulated territorial intrusion (higher STI-PC2 scores), such that they

responded quicker to playback and would counter-sing more often; male STI-PC2 scores were higher than those of females, suggesting that males may drive this component. When corrected for multiple correlations, we also failed to detect a relationship between the unmanipulated coloration of the crown, the scapular, and the contrast between the two patches, further supporting the null expectation that female scapular coloration is not a signal in aggressive contexts. However, when these tests were unadjusted, we did find that there appears to be some weak trends between behavior and color; that these disappeared when adjusting for multiple tests may suggest these are spurious. We may speculate that perhaps this also points to a contemporary function of female coloration, although that function is unclear at present.

Selection should favor song for long-range broadcasting and plumage elaboration for close encounters (Webb et al. 2016). Mirror image assays isolate confounding stimuli and present focal individuals with challenges of equivalent quality directly in front of them, thus providing the most direct route to explore color function. Leitão et al. (2019a) employed a similar experiment in lovely fairywrens (*Malurus amabilis*) but found opposite behavioral patterns depending on if they were testing natural versus experimentally manipulated coloration. Female and male lovely fairywrens were more aggressive toward mirror reflections that were naturally less ornamented but were also more aggressive toward mirror reflections that had experimentally increased cheek coloration. Their results suggest that ornaments operate as signals in competitive contexts in both sexes of this species, as well as showing how increased aggression may be an enforcement mechanism to prevent dishonest signaling (Leitão et al. 2019a). In contrast, we found no relationship between aggression and experimental plumage manipulation nor the melanin-based crown coloration, structural white coloration, or the achromatic contrast between these two patches during our correlative analysis. Our previous work in this system suggests that song may have a disproportionate effect on eliciting aggressive territorial responses (Jones et al. 2021) in aggressive contexts. However, the relationship between among-individual variation in song quality and aggressive behaviors has yet to be tested and remains unclear.

An appreciation for female signals and the processes underlying their evolution has recently begun to receive long-overdue scientific attention. Indeed, thanks to a series of insightful papers, it is now better appreciated how female-specific selection promotes elaborate signals in manners that may, or may not, be analogous to that of male animals (LeBas 2006; Clutton-Brock 2007; Tobias et al. 2012; Cain and Rosvall 2014; Odom et al. 2014, 2021; Dale et al. 2015; Webb et al. 2016; Riebel et al. 2019; Doutrelant et al. 2020). Female ornamentation is often

thought to be associated with selection for non-sexual, ecological resources (i.e., intrasexual competition) rather than mate choice per se (e.g., Heinsohn et al. 2005; LeBas 2006; Rosvall 2011; Tobias et al. 2012). However, we fail to support this hypothesis in a subspecies of white-shouldered fairywrens where all contemporary females naturally express plumage ornamentation. It may be that rather than a signal toward rival females of resource holding potential, female coloration may act as a sexual ornament to males, indicative of their phenotypic and/or genetic quality (Amundsen and Forsgren 2001; Weiss 2006; Fitzpatrick and Servedio 2017, 2018). Or perhaps the trait evolved in response to increased competition as hypothesized but has since lost this function. These possibilities remain untested, emphasizing that continued work is required to better resolve the adaptive significance and contemporary function of female ornamentation in this system.

Data availability Data and associated R scripts are available via Dryad: DOI:10.5061/dryad.6djh9w12k.

Declarations

Ethics approval Our study was carried out in strict accordance with the guidelines established by the Tulane University Institutional Animal Care and Use Committee (#0395R2) as well as in adherence to research permits from the Conservation and Environment Protection Authority of Papua New Guinea (#99902100765). All birds were captured, processed, exposed to one (or two) mirror assays, and then released in under one hour. We continuously monitored our mist-nets and removed birds immediately upon hitting the net. All individuals involved in the removal of birds from mist-nets were trained in the appropriate way to handle birds prior to their handling. All manipulated birds recaptured had marker residue cleaned with rubbing alcohol and feathers plucked to induce healthy molting of a normal shoulder patch. Through routine monitoring of the birds throughout the season, we confirmed that the plumage manipulation did not have any lasting effects (i.e., shoulders were visibly naturally white again a few weeks after treatment).

Conflict of interest The authors declare no competing interests.

References

Amundsen T, Forsgren E (2001) Male mate choice selects for female coloration in a fish. P Natl Acad Sci USA 98:13155–13160. https://doi.org/10.1073/pnas.211439298

Audacity Team (2018) Audacity(R), v 2.2.2, https://audacityteam.org/

Autumn K, Ryan MJ, Wake DB (2002) Integrating historical and mechanistic biology enhances the study of adaptation. Q Rev Biol 77:383–408. https://doi.org/10.1086/344413

Bates D, Mächler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48. https://doi.org/10.18637/jss.v067.i01

Beck ML, Hopkins WA (2019) The relationship between plumage coloration and aggression in female tree swallows. J Avian Biol 50:e01910. https://doi.org/10.1111/jav.01910

Berzins LL, Dawson RD (2018) Experimentally altered plumage brightness of female tree swallows (*Tachycineta bicolor*) influences nest site retention and reproductive success. Can J Zool 96:600–607. https://doi.org/10.1139/cjz-2017-0156

Biernaskie JM, Grafen A, Perry JC (2014) The evolution of index signals to avoid the cost of dishonesty. Proc R Soc B 281:20140876. https://doi.org/10.1098/rspb.2014.0876

Boersma J, Enbody ED, Jones JA, Nason D, Lopez-Contreras KJ, 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

Bonduriansky R (2001) The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol Rev 76:305–339. https://doi.org/10.1017/S1464793101005693

Cain KE, Rosvall KA (2014) Next steps for understanding the selective relevance of female-female competition. Front Ecol Evol 2:32. https://doi.org/10.3389/fevo.2014.00032

Cardoso GC, Mota PG (2010) Evolution of female carotenoid coloration by sexual constraint in Carduelis finches. BMC Evol Biol 10:82. https://doi.org/10.1186/1471-2148-10-82

Cassey P, Ewen JG, Blackburn TM, Hauber ME, Voryobyev M, Marshall NJ (2008) Eggshell colour does not predict measures of maternal investment in eggs of *Turdus* thrushes. Naturwissenschaften 95:713–721. https://doi.org/10.1007/s00114-008-0376-x

Chaine AS, Shizuka D, Block TA, Zhang L, Lyon BE (2018) Manipulating badges of status only fools strangers. Ecol Lett 21:1477–1485. https://doi.org/10.1111/ele.13128

Clutton-Brock T (2007) Sexual selection in males and females. Science 318:1882–1885. https://doi.org/10.1126/science.1133311

Cockrem JF, Silverin B (2002) Variation within and between birds in corticosterone responses of great tits (*Parus major*). Gen Comp Endocrinol 125:197–206. https://doi.org/10.1006/gcen.2001.7750

Cole GL, Endler JA (2016) Male courtship decisions are influenced by light environment and female receptivity. Proc R Soc B 283:20160861. https://doi.org/10.1098/rspb.2016.0861

Courtiol A, Etienne L, Feron R, Godelle B, Rousset F (2016) The evolution of mutual mate choice under direct benefits. Am Nat 188:521–538. https://doi.org/10.1086/688658

Cuthill IC (2006) Color perception. In: Hill GE, McGraw KJ (eds) Bird Coloration I: Mechanisms and Measurements. Harvard University Press, Cambridge, MA, pp 3–40

Dale J, Dey C, Delhey K, Kempenaers B, Valcu M (2015) The effects of life-history and social selection on male and female plumage coloration. Nature 527:367–370. https://doi.org/10.1038/nature15509

Delhey K, Delhey V, Kempenaers B, Peters A (2015) A practical framework to analyze variation in animal colors using visual models. Behav Ecol 26:367–375. https://doi.org/10.1093/beheco/aru198

Delhey K, Hall M, Kingma SA, Peters A (2013) Increased conspicuousness can explain the match between visual sensitivities and blue plumage colours in fairy-wrens. Proc R Soc B 280:20121771. https://doi.org/10.1098/rspb.2012.1771

Delhey K, Peters A (2008) Quantifying variability of avian colours: are signalling traits more variable? PLoS ONE 3:e1689. https://doi.org/10.1371/journal.pone.0001689

Diamant ES, Falk JJ, Rubenstein DR (2021) Male-like female morphs in hummingbirds: the evolution of a widespread sex-limited plumage polymorphism. Proc R Soc B 288:20203004. https://doi.org/10.1098/rspb.2020.3004

Doutrelant C, Fargevieille A, Grégoire A (2020) Evolution of female coloration: what have we learned from birds in general and blue tits in particular. Adv Stud Behav 52:123–202. https://doi.org/10.1016/bs.asb.2020.03.001

Driskell AC, Norman JA, Pruett-Jones S, Mangall E, Sonsthagen S, Christidis L (2011) A multigene phylogeny examining evolutionary and ecological relationships in the Australo-Papuan wrens of the subfamily Malurinae (Aves). Mol Phylogenet Evol 60:480–485. https://doi.org/10.1016/j.ympev.2011.03.030

Enbody ED, Boersma J, Jones JA et al (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 ED, 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

Endler JA, Mielke PW (2005) Comparing entire colour patterns as birds see them. Biol J Linn Soc 86:405–431. https://doi.org/10.1111/j.1095-8312.2005.00540.x

Ferns PN, Hinsley SA (2004) Immaculate tits: head plumage pattern as an indicator of quality in birds. Anim Behav 67:261–272. https://doi.org/10.1016/j.anbehav.2003.05.006

Fitzpatrick CL, Servedio MR (2017) Male mate choice, male quality, and the potential for sexual selection on female traits under polygyny. Evolution 71:174–183. https://doi.org/10.1111/evo.13107

Fitzpatrick CL, Servedio MR (2018) The evolution of male mate choice and female ornamentation: a review of mathematical models. Curr Zool 64:323–333. https://doi.org/10.1093/cz/zoy029

Fitzpatrick S, Berglund A, Rosenqvist G (1995) Ornaments or offspring: costs to reproductive success restrict sexual selection processes. Biol J Linn Soc 55:251–260. https://doi.org/10.1111/j.1095-8312.1995.tb01063.x

Freeman BG, Scholer MN, Boehm MMA, Heavyside J, Schluter D (2020) Adaptation and latitudinal gradients in species interactions: nest predation in birds. Am Nat 196:E160–E166. https://doi.org/10.1086/711415

Gallup GG Jr (1968) Mirror-image stimulation. Psychol Bull 70:782–793. https://doi.org/10.1037/h0026777

Gould SJ, Vrba ES (1982) Exaptation – a missing term in the science of form. Paleobiology 8:4–15. https://doi.org/10.2307/2400563

Griggio M, Valera F, Casas A, Pilastro A (2005) Males prefer ornamented females: a field experiment of male choice in the rock sparrow. Anim Behav 69:1243–1250. https://doi.org/10.1016/j.anbehav.2004.10.004

Hall ML, Peters A (2008) Coordination between the sexes for territorial defence in a duetting fairy-wren. Anim Behav 76:65–73. https://doi.org/10.1016/j.anbehav.2008.01.010

Hart NS (2001) Variations in cone photoreceptor abundance and the visual ecology of birds. J Comp Physiol A 187:685–697. https://doi.org/10.1007/s00359-001-0240-3

Hegyi G, Kötél D, Laczi M (2015) Direct benefits of mate choice: a meta-analysis of plumage colour and offspring feeding rates in birds. Sci Nat 102:62. https://doi.org/10.1007/s00114-015-1311-6

Heinsohn R, Legge S, Endler JA (2005) Evolution: extreme reversed sexual dichromatism in a bird without sex role reversal. Science 309:617–619. https://doi.org/10.1126/science.1112774

Hill GE, McGraw KJ (eds) (2006) Bird Coloration, Vol 1: Mechanisms and Measurements. Harvard University Press, Cambridge, MA

Hofmann CM, Cronin TW, Omland KE (2008) Evolution of sexual dichromatism. 1. Convergent losses of elaborate female coloration in new world orioles (*Icterus* spp.). Auk 125:790–795. https://doi.org/10.1525/auk.2008.07113

Jawor JM, Gray N, Beall SM, Breitwisch R (2004) Multiple ornaments correlate with aspects of condition and behaviour in female northern cardinals, *Cardinalis cardinalis*. Anim Behav 67:875–882. https://doi.org/10.1016/j.anbehav.2003.05.015

Johnson AE, Jordan Price J, Pruett-Jones S (2013) Different modes of evolution in males and females generate dichromatism in fairy-wrens (Maluridae). Ecol Evol 3:3030–3046. https://doi.org/10.1002/ece3.686

Johnstone RA, Norris K (1993) Badges of status and the cost of aggression. Behav Ecol Sociobiol 32:127–134

Jones JA, Odom KJ, Hoppe IR, Nason D, Ketaloya S, Karubian J (2021) Correlated evolution of distinct signals associated with increased social selection in female white-shouldered fairywrens. Ecol Evol 11:17352–17363. https://doi.org/10.1002/ece3.8370

Karubian J (2013) Female ornamentation in *Malurus* fairy-wrens: a hidden evolutionary gem for understanding female perspectives on social and sexual selection. Emu 113:248–258. https://doi.org/10.1071/MU12093

Karubian J, Swaddle JP, Varian-Ramos CW, Webster MS (2009) The relative importance of male tail length and nuptial plumage on social dominance and mate choice in the red-backed fairy-wren *Malurus melanocephalus*: evidence for the multiple receiver hypothesis. J Avian Biol 40:559–568. https://doi.org/10.1111/j.1600-048X.2009.04572.x

Kelly CD, Godin J-GJ (2001) Predation risk reduces male-male sexual competition in the Trinidadian guppy (*Poecilia reticulata*). Behav Ecol Sociobiol 51:95–100. https://doi.org/10.1007/s002650100410

Kraaijeveld K (2014) Reversible trait loss: the genetic architecture of female ornaments. Annu Rev Ecol Evol S 45:159–177. https://doi.org/10.1146/annurev-ecolsys-120213-091550

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

LeBas NR (2006) Female finery is not for males. Trends Ecol Evol 21:170–173. https://doi.org/10.1016/j.tree.2006.01.005

LeBas NR, Hockham LR, Ritchie MG (2003) Nonlinear and correlational sexual selection on "honest" female ornamentation. Proc R Soc Lond B 270:2159–2165. https://doi.org/10.1098/rspb.2003.2482

Leitão AV, Hall ML, Delhey K, Mulder RA (2019a) Female and male plumage colour signals aggression in a dichromatic tropical songbird. Anim Behav 150:285–301. https://doi.org/10.1016/j.anbehav.2019.01.025

Leitão AV, Hall ML, Venables B, Mulder RA (2019b) Ecology and breeding biology of a tropical bird, the Lovely Fairy-Wren (*Malurus amabilis*). Emu 119:1–13. https://doi.org/10.1080/01584197.2018.1498745

Lipshutz SE, Rosvall KA (2021) Nesting strategy shapes territorial aggression but not testosterone: a comparative approach in

female and male birds. Horm Behav 133:104995. https://doi.org/10.1016/j.yhbeh.2021.104995

López-Idiáquez D, Vergara P, Fargallo JA, Martínez-Padilla J (2016) Female plumage coloration signals status to conspecifics. Anim Behav 121:101–106. https://doi.org/10.1016/j.anbehav.2016.08.020

Maia R, Gruson H, Endler JA, White TE (2019) pavo 2: New tools for the spectral and spatial analysis of colour in R. Methods Ecol Evol 10:1097–1107. https://doi.org/10.1111/2041-210X.13174

Mason NA, Bowie RCK (2020) Plumage patterns: ecological functions, evolutionary origins, and advances in quantification. Auk 137:ukaa060. doi: https://doi.org/10.1093/auk/ukaa060

Maynard Smith J, Harper DG (1988) The evolution of aggression: can selection generate variability? Phil Trans R Soc B 319:557–570. https://doi.org/10.1098/rstb.1988.0065

Maynard Smith J, Price GR (1973) The logic of animal conflict. Nature 246:15–18. https://doi.org/10.1038/246015a0

Medina I, Delhey K, Peters A, Cain KE, Hall ML, Mulder RA, Langmore NE (2017) Habitat structure is linked to the evolution of plumage colour in female, but not male, fairy-wrens. BMC Evol Biol 17:35. https://doi.org/10.1186/s12862-016-0861-3

Midamegbe A, Grégoire A, Perret P, Doutrelant C (2011) Female-female aggressiveness is influenced by female coloration in blue tits. Anim Behav 82:245–253. https://doi.org/10.1016/j.anbehav.2011.04.020

Montgomerie R (2006) Analyzing colors. In: Hill GE, McGraw KJ (eds) Bird Coloration I: Mechanisms and Measurements. Harvard University Press, Cambridge, MA, pp 90–147

Murphy TG, Rosenthal MF, Montgomerie R, Tarvin KA (2009) Female American goldfinches use carotenoid-based bill coloration to signal status. Behav Ecol 20:1348–1355. https://doi.org/10.1093/beheco/arp140

Nordeide JT, Kekäläinen J, Janhunen M, Kortet R (2013) Female ornaments revisited – are they correlated with offspring quality? J Anim Ecol 82:26–38. https://doi.org/10.1111/1365-2656.12021

Ödeen A, Håstad O (2013) The phylogenetic distribution of ultraviolet sensitivity in birds. BMC Evol Biol 13:36

Ödeen A, Pruett-Jones S, Driskell AC, Armenta JK, Håstad O (2012) Multiple shifts between violet and ultraviolet vision in a family of passerine birds with associated changes in plumage coloration. Proc R Soc Lond B 279:1269–1276. https://doi.org/10.1098/rspb.2011.1777

Odom KJ, Cain KE, Hall ML et al (2021) Sex role similarity and sexual selection predict male and female song elaboration and dimorphism in fairy-wrens. Ecol Evol 11:17901–17919. https://doi.org/10.1002/ece3.8378

Odom KJ, Hall ML, Riebel K, Omland KE, Langmore NE (2014) Female song is widespread and ancestral in songbirds. Nat Commun 5:3379. https://doi.org/10.1038/ncomms4379

Olsson P, Lind O, Kelber A (2018) Chromatic and achromatic vision: parameter choice and limitations for reliable model predictions. Behav Ecol 29:273–282. https://doi.org/10.1093/beheco/arx133

Omland KE, Hofmann CM (2006) Adding color to the past: ancestral-state reconstruction of coloration. In: Hill GE, McGraw KJ (eds) Bird Coloration II: Function and Evolution. Harvard University Press, Cambridge, MA, pp 417–454

Parker GA (1974) Assessment strategy and the evolution of fighting behaviour. J Theor Biol 47:223–243. https://doi.org/10.1016/0022-5193(74)90111-8

R Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/.

Revelle W (2021) psych: procedures for psychological, psychometric, and personality research. R package v2.1.6, https://personality-project.org/r/psych/

Riebel K, Odom KJ, Langmore NE, Hall ML (2019) New insights from female bird song: towards an integrated approach to studying Male and female communication roles. Biol Lett 15:20190059. https://doi.org/10.1098/rsbl.2019.0059

Rohwer S (1982) The evolution of reliable and unreliable badges of fighting ability. Am Zool 22:531–546. https://doi.org/10.1093/icb/22.3.531

Rosvall KA (2011) Intrasexual competition in females: evidence for sexual selection? Behav Ecol 22:1131–1140. https://doi.org/10.1093/beheco/arr106

Rowley I, Russell E (1997) Bird families of the world: fairy-wrens and Grasswrens. Oxford University Press, Oxford, UK

Rubenstein DR, Lovette IJ (2009) Reproductive skew and selection on female ornamentation in social species. Nature 462:786–789. https://doi.org/10.1038/nature08614

Santos ESA, Scheck D, Nakagawa S (2011) Dominance and plumage traits: meta-analysis and metaregression analysis. Anim Behav 82:3–19. https://doi.org/10.1016/j.anbehav.2011.03.022

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675. https://doi.org/10.1038/nmeth.2089

Senar JC (2006) Bird colors as intrasexual signals of aggression and dominance. In: Hill GE, McGraw KJ (eds) Bird Coloration II: Function and Evolution. Harvard University Press, Cambridge, MA, USA, pp 125–193

Slater PJB, Mann NI (2004) Why do the females of many bird species sing in the tropics? J Avian Biol 35:289–294. https://doi.org/10.1111/j.0908-8857.2004.03392.x

Stockley P, Bro-Jørgensen J (2011) Female competition and its evolutionary consequences in mammals. Biol Rev 86:341–366. https://doi.org/10.1111/j.1469-185X.2010.00149.x

Stutchbury BJM, Morton ES (2001) Behavioral ecology of tropical birds. Academic Press, London, UK

Stutchbury BJM, Morton ES (2008) Recent advances in the behavioral ecology of tropical birds. Wilson J Ornithol 120:26–37. https://doi.org/10.1676/07-018.1

Swaddle JP, Karubian J, Pruett-jones S (2000) A novel evolutionary pattern of reversed sexual dimorphism in fairy wrens: implications for sexual selection. Behav Ecol 11:345–349. https://doi.org/10.1093/beheco/11.3.345

Taff CC, Zimmer C, Scheck D, Ryan TA, Houtz JL, Smee MR, Hendry TA, Vitousek MN (2021) Plumage manipulation alters associations between behaviour, physiology, the internal microbiome and fitness. Anim Behav 178:11–36. https://doi.org/10.1016/j.anbehav.2021.05.012

Templeton CN, Akçay Ç, Campbell SE, Beecher MD (2012) Soft song is a reliable signal of aggressive intent in song sparrows. Behav Ecol Sociobiol 66:1503–1509. https://doi.org/10.1007/s00265-012-1405-5

Tobias JA, Montgomerie R, Lyon BE (2012) The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. Phil Trans R Soc B 367:2274–2293. https://doi.org/10.1098/rstb.2011.0280

Vorobyev M, Osorio D (1998) Receptor noise as a determinant of colour thresholds. Proc R Soc Lond B 265:351–358. https://doi.org/10.1098/rspb.1998.0302

Vorobyev M, Osorio D, Bennett AT, Marshall NJ, Cuthill IC (1998) Tetrachromacy, oil droplets and bird plumage colours. J Comp Physiol A 183:621–633. https://doi.org/10.1007/s003590050286

Weaver RJ, Koch RE, Hill GE (2017) What maintains signal honesty in animal colour displays used in mate choice? Phil Trans R Soc B 372:20160343. https://doi.org/10.1098/rstb.2016.0343

Webb WH, Brunton DH, Aguirre JD, Thomas DB, Valcu M, Dale J (2016) Female song occurs in songbirds with more elaborate female coloration and reduced sexual dichromatism. Front Ecol Evol 4:22. https://doi.org/10.3389/fevo.2016.00022

Weiss SL (2006) Female-specific color is a signal of quality in the striped plateau lizard (*Sceloporus virgatus*). Behav Ecol 17:726–732. https://doi.org/10.1093/beheco/arl001

West-Eberhard MJ (1979) Sexual selection, social competition, and evolution. P Am Philos Soc 123:222–234

West-Eberhard MJ (1983) Sexual selection, social competition, and speciation. Q Rev Biol 58:155–183

Wiens JJ (2001) Widespread loss of sexually selected traits: how the peacock lost its spots. Trends Ecol Evol 16:517–523. https://doi.org/10.1016/S0169-5347(01)02217-0

Wilkins MR, Odom KJ, Benedict L, Safran RJ (2020) Analysis of female song provides insight into the evolution of sex differences in a widely studied songbird. Anim Behav 168:69–82. https://doi.org/10.1016/j.anbehav.2020.07.018