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Neuroendocrinology of sex-role reversal

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Abstract

Females of some species are considered sex-role reversed, meaning that they face stronger competition for mates compared to males. While much attention has been paid to behavioral and morphological patterns associated with sex-role reversal, less is known about its physiological regulation. Here, we evaluate activational and organizational hypotheses relating to the neuroendocrine basis of sex-role reversal. We refute the most widely tested activational hypothesis for sex differences in androgen secretion; sex-role reversed females do not have higher levels of androgens in circulation than males. However, we find some evidence that the activational effects of androgens may be sex-specific; circulating androgen levels correlate with some competitive phenotypes in sex-role reversed females. Organizational effects may explain these relationships, considering that early exposure to sex steroids can shape later sensitivity to hormones, often in sex-specific ways. We review evidence that sex-role reversed females have higher tissue-specific sensitivity to androgens than males, at least in some species and tissues. Moving forward, experimental and correlative studies on the ontogeny and expression of sex-role reversal will help reveal the mechanisms that generate sex-specific behaviors and sex roles.

35 “Why are males masculine, females feminine and occasionally vice-versa?”

36 – GC Williams, 1975

38 **An Introduction to Sex-Role Reversal**

39 Why are females and males different? Biologists have been trying to answer this question
40 since Darwin first postulated that ‘instinct’ (behavior) may be shaped by natural selection (1859).
41 In his Victorian era, male animals were considered dominant and promiscuous, whereas female
42 animals were thought to be coy and subdued. In the intervening years, we have learned a lot
43 about ‘sex roles’ across the animal kingdom. For many species, as Darwin and his
44 contemporaries observed, males face stronger competition for mating opportunities than females,
45 and females tend to conduct the majority of parental care (Darwin 1871; Clutton-Brock 1991;
46 Andersson 1994). Whereas territorial aggression and promiscuity were historically considered
47 male traits, however, behavioral ecologists now recognize that intrasexual competition and
48 multiple-mating are adaptive and widespread behaviors in females of many species (Clutton-
49 Brock 2009; Rosvall 2011; Hare and Simmons 2018). Sex-role reversal (SRR) occurs when
50 sexual selection among females is stronger than sexual selection acting among males (Vincent
51 et al. 1992; Kvarnemo and Ahnesjö 1996). SRR females are characterized by phenotypes
52 typically associated with males, including morphological traits like heavier body mass, larger
53 weaponry, and more ornamentation, as well as behavioral traits like higher territorial aggression
54 or more intense courtship rituals. These traits are thought to facilitate female-female competition
55 for mates and breeding territories (Emlen and Oring 1977; Gwynne 1991).

56 Classic work by Bateman (1948) and Trivers (1972) attributed the evolution of sex roles
57 to anisogamy, although this has proven challenging to reconcile with sex-role reversal. In
58 general, male gametes (sperm) are smaller and more numerous than nutrient-rich female
59 gametes (ova), and so males may be more available to mate than females, who may be
60 predisposed to caring for their offspring based on this initial asymmetry in parental investment.

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3 61 For decades, this anisogamy argument dominated theory on the evolution of sex roles: sex
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5 62 differences in initial parental investment drive the degree of mating competition and direction of
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7 63 sexual selection (reviewed in Hoquet, 2020). However, anisogamy cannot explain the evolution of
8
9 64 SRR because SRR-females are nonetheless female; they always produce the larger gamete,
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11 65 even when they compete more than males. Recent theoretical work indicates that additional
12
13 66 factors including multiple paternity, adult mortality, and sex ratios may generate co-evolutionary
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15 67 feedbacks that influence the strength and sex-specificity of sexual selection (Kokko and
16
17 68 Jennions 2008; Fromhage and Jennions 2016). Despite these advances to our understanding of
18
19 69 the ultimate drivers of SRR, much less is known about the proximate mechanisms that give rise
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21 70 to SRR.

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24 71 Sex steroids are logical candidates for the physiological regulation of SRR, because
25
26 72 these hormones are associated with many sexually dimorphic traits, especially those related to
27
28 73 mating and mating competition (Adkins-Regan 2005). Sex steroids act in two main ways, the
29
30 74 first of which operates early in life during a critical period when exposure to a hormone (or lack
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32 75 thereof) can permanently organize tissue structure and function i.e. *organizational effects*
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34 76 (Phoenix et al. 1959; Arnold and Breedlove 1985), which determine whether later exposure to a
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36 77 hormone can bring about a phenotypic effect. One of the primary modes of action for
37
38 78 organizational effects is to change the anatomical distribution and/or abundance of sex steroid
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40 79 receptors, early in the life and often lasting into adulthood (Moore et al. 1998). Activational
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42 80 effects typically occur during adulthood, when animals change aspects of their phenotype in
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44 81 response to changing hormone levels in circulation. For sex steroids, hormone secretion is
45
46 82 regulated by the hypothalamic-gonadal-pituitary (HPG) axis, when external stimuli prompt the
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48 83 hypothalamus to secrete gonadotropin-releasing hormone (GnRH). GnRH then stimulates the
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50 84 pituitary to release gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone
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52 85 (FSH) into the bloodstream. These gonadotropins signal to the gonads to initiate gametogenesis
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54 86 as well as produce sex steroids including estrogen, progesterone, and testosterone, as well as
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11-ketotestosterone in fishes (Schulz et al. 2010). Many researchers have found important connections between sex steroids, their cellular mechanisms of action, and the evolution of sexually selected traits, linking the physiological origins of diversity in mating phenotypes across animals (Wingfield et al. 1990; Soma 2006; Fuxjager and Schuppe 2018; Lipshutz et al. 2019; Cox 2020). Critically, these mechanisms operate in both sexes (Staub and De Beer 1997), providing the opportunity to investigate how variation in sex steroid signaling may contribute to the origin and expression of SRR.

Here, we evaluate two key hypotheses on the role of sex steroids in the evolution of SRR. First, we examine the evidence that SRR is explained by activational effects of sex steroid secretion, focusing on sex differences in levels of androgens in circulation, as well as co-variation between androgens and competitive phenotypes in SRR species. Next, we assess hypotheses relating to the organizational basis of SRR, asking whether androgen exposure early in development may explain SRR behavior and morphology, and/or sex differences in tissue-level sensitivity to androgens (e.g. androgen receptor abundance). We focus primarily on SRR birds and fishes, which have received the most attention to date, and we draw inferences from species with conventional sex roles.

H1: SRR stems from activational effects of sex steroids

Do females and males differ in androgen levels in circulation?

Two decades ago, Eens and Pinxten (2000) reviewed studies from field endocrinology to evaluate the hypothesis that SRR females have male-typical physiological mechanisms, specifically that testosterone secretion may be higher in SRR females, a reversal from the conventional pattern. Evidence from three SRR avian species did not support this hypothesis: during mating competition and courtship, males have higher testosterone in circulation compared with females (spotted sandpiper (*Actitis macularius*), Rissman and Wingfield 1984; Wilson's phalarope (*Phalaropus tricolor*), Fivizzani et al. 1986; red-necked phalarope

(*Phalaropus lobatus*), Gratto-Trevor et al. 1990), reflecting patterns seen in species with conventional sex roles.

To re-evaluate this hypothesis 20 years later, we conducted a meta-analysis on sex differences in circulating androgens in SRR species, including both testosterone and its more potent metabolite 5-alpha dihydrotestosterone (DHT), both of which bind to the androgen receptor. For the six SRR avian species with relevant data, we compiled mean androgen levels from HormoneBase (Vitousek et al. 2018) or the primary literature, using data reported in the text or measured from figures using WebPlotDigitizer (Rohatgi 2019) (Supplementary File 1). Our analysis therefore updates Eens and Pinxten's findings with more recent studies on androgen secretion in SRR avian species including black coucals (*Centropus grillii*), barred buttonquails (*Turnix suscitator*), and northern jacanas (*Jacana spinosa*) (Goymann and Wingfield 2004; Voigt and Goymann 2007; Voigt 2016; Lipshutz and Rosvall 2020). To estimate the standardized effect size of sex differences in androgens across SRR species, we used random effects models in the package *metafor* in R (Viechtbauer 2010). We ran separate models comparing females to males in different breeding stages (i.e. courting vs. caring), as male androgen levels typically decline with parental care (Wingfield et al. 1990). We also ran a mixed effects model with breeding stage as a modulator, to compare the influence of breeding stage on sex differences in levels of androgens in circulation.

First, focused on data from females and males, sampled when both sexes were competing and courting, our analysis showed an average negative and significant effect size ($\mu = -1.14$, $z = -5.24$, $p < 0.0001$), indicating that SRR males have higher androgens than females (Figure 1). Thus, during the period of time when both sexes are seeking mates, SRR females secrete androgens in ways that are similar to females of species with conventional sex roles, and SRR males follow patterns similar to males with conventional roles. However, this courtship-stage model had significant heterogeneity in sex differences ($I^2 = 0.56$, $Q = 22.23$, $df =$

10, $p = 0.014$), indicating that not every species demonstrated the same pattern. For example, female and male barred buttonquails did not differ in levels of DHT in circulation (Voigt 2016).

When we analyzed androgen levels in SRR birds during the period of time when males are parenting, however, the effect size was not significant ($\mu = -0.019$, $z = -0.14$, $p = 0.89$), indicating no overall sex difference in circulating androgen levels (Figure 1). This parental-stage model did not have significant heterogeneity in sex differences ($I^2 = 0$, $Q = 4.81$, $df = 7$, $p = 0.68$), indicating similar patterns across species. During the parental care phase of breeding, these SRR males and females did not differ significantly in levels of testosterone and DHT in circulation, meaning the sex differences in androgen levels seen during courtship are ablated during periods of male parental care. Indeed, direct comparison of the courtship-stage and parental-stage data into a single model shows that breeding stage explained 79% of the variation in effect size ($Q_M = 18.52$, $df = 1$, $p < 0.0001$). In males with conventional sex roles, androgen levels decline during parental care, though not typically to female-like levels (Wingfield et al. 1990; Hirschenhauser and Oliveira 2006). Our finding – that androgens in SRR males fall to levels as low as females during parental care – may represent an extreme reduction in androgen sexual dimorphism, potentially suggesting that SRR drives lower T levels in males, higher T levels in females, or some combination of the two (sensu Goymann and Wingfield 2014). We find it interesting that in SRR species, circulating androgen levels are most similar between the sexes during the breeding stage when the sexes are most behaviorally divergent, i.e. female competition and male parental care. Clearly, sex differences in levels of androgens in circulation alone do not explain SRR.

Beyond T and DHT, fewer studies have measured other sex steroids or prohormones in SRR species, and evidence to date is mixed as to whether secretion of these hormones is sexually dimorphic. For example, a study of black coucals suggests that secretion of the androgenic precursors androstenedione and DHEA is similar between the sexes, regardless of breeding stage (Goymann and Wingfield 2004). Estradiol levels are typically higher in females

than males in conventional species, a pattern that is reflected in some SRR species (Fivizzani et al. 1986), but not others, for which estradiol levels are similar between the sexes, or higher than expected in SRR males (Rissman and Wingfield 1984; Goymann and Wingfield 2004; Voigt 2016). Progesterone levels are also typically higher in females than males, which is reflected in some SRR species (Fivizzani et al. 1986; Gratto-Trevor et al. 1990), but not others (Voigt 2016). In SRR broadnosed pipefish (*Syngnathus typhle*) and greater pipefish (*Syngnathus acus*), breeding males have higher 11-ketotestosterone than brooding males (Mayer et al. 1993), a pattern reflected in non-SRR teleost fish (Knapp et al. 1999; Mayer et al. 2004), but circulating hormones have been challenging to measure in sygnathids (Scobell and Mackenzie 2011). Together, these studies suggest that the sex steroid profiles of SRR females and males are similar to their counterparts with conventional sex roles, largely confirming the conclusions of Eens and Pinxten (2000).

Are activational effects of androgens sexually dimorphic?

Androgen levels themselves are only part of the regulation of androgen-mediated phenotypes, and there is good evidence that the sexes may differ in their gene regulatory responses to androgens (Van Nas et al. 2009; Peterson et al. 2014). Thus, androgens may differentially affect females and males in SRR species, even when hormone levels themselves largely follow patterns of sexual dimorphism seen in species with conventional sex roles. Sex-specific activational effects of androgens can be assessed with (a) correlations that directly link SRR behaviors and morphological traits with sex steroids, or (b) experimental treatments between sex steroids and traits of interest.

One approach to understand the physiological regulation of SRR is to link individual variation in endocrine phenotypes directly with variation in competitive traits, including ornamentation, weaponry, and body size. Correlational support for this idea does exist in SRR species, although it is quite limited. For instance, levels of testosterone in circulation positively

correlate with melanin throat patch and body condition in female barred buttonquails (Muck and Goymann 2011). In female northern jacanas, testosterone secretion positively correlates with the size of weaponry, wing spurs, but this relationship was not found in males (Lipshutz and Rosvall 2020). Thus, despite low levels of testosterone in circulation in SRR females, there is some evidence that testosterone is related to the regulation of competitive traits in SRR females in some way. Other studies find that non-steroid hormones may regulate competitive traits in relation to SRR. In the two-spotted goby (*Gobiomacdonatus flavescens*), a species with dynamic sex roles that change from conventional to reversed (Forsgren et al. 2004), pigmentation of the female belly ornament is regulated by the pituitary hormone prolactin as well as alpha-melanocyte stimulating hormone, but this ornament does not appear to be regulated by the sex steroids testosterone, 11-keto-testosterone, or estradiol in this example (Sköld et al. 2008).

Experimental data to directly address sex-specific activational effects of androgens in SRR species is also limited, but indirect evidence suggests that both morphological and behavioral traits involved in mating competition respond to experimental manipulation of sex steroids. Early work in the SRR Wilson's and Red-necked phalaropes, for which females have brighter nuptial plumage, found that exogenous testosterone induces nuptial plumage in both females and males, suggesting that nuptial feather growth is androgen-dependent (Johns 1964). In male gulf pipefish (*Syngnathus scovelli*), exposure to estrogen impacts the development of the iridescent transverse band (Partridge et al. 2010), a sexually selected ornament in females (Flanagan et al. 2014). These findings parallel work in species with conventional roles, for which testosterone implants in females increase male-typical traits including courtship displays (Day et al. 2007), vocalizations (Nottebohm 1980; Chiver and Schlinger 2019), and nuptial plumage (Lindsay et al. 2016). In other words, the activational effects of sex steroids can reverse sex-specific phenotypes in SRR males in a manner similar to non-SRR females. For competitive behaviors like aggression, the link with testosterone in SRR species has mixed support. In female barred buttonquails, implantation with testosterone did not

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3 216 increase aggression, and territorial challenge decreased levels of testosterone in circulation
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5 217 (Muck and Goymann 2019). In female black coucals, testosterone did not differ between
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7 218 challenged and unchallenged females, but territorial challenge decreased levels of progesterone
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9 219 in circulation, and progesterone implants reduced female aggression (Goymann et al. 2008).
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11 220 Similarly, the lack of a relationship between testosterone and aggressive behavior is reflected in
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13 221 many species with conventional sex roles (Wingfield et al. 2019). In sum, variation in androgens
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15 222 in circulation may explain some competitive phenotypes but not others, and these relationships
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17 223 can vary by sex, suggesting we must also look beyond activational hypotheses on the origin and
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19 224 expression of SRR.
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24 226 **H2: SRR stems from organizational effects of sex steroids**

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26 227 Organizational effects of sex steroids deliver some promise as proximate regulators of
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28 228 SRR (Adkins-Regan 2012). An ontogenetic hypothesis for SRR was proposed by Fivizzani et al.
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30 229 (1986), drawing from observations in many species that exposure to sex steroid hormones early
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32 230 in development can generate sex differences in later responsiveness to these hormones in
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34 231 adulthood (Arnold 2009). For instance, exposure to high testosterone early in life, in utero, or in
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36 232 ovo can influence suites of sexual characteristics (vom Saal and Bronson 1980; Hotchkiss et al.
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38 233 2007), a phenotypic effect that is at least partly mediated by tissue level changes in sensitivity to
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40 234 sex steroids (Mori et al. 2010; Pfannkuche et al. 2011). Sensitivity is comprised of a number of
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42 235 factors, including sex steroid receptors, as well as enzymes that produce steroid hormones and
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44 236 convert them into more or less active forms (Ball and Balthazart 2008). In particular,
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46 237 testosterone can be locally converted by the enzymes aromatase and 5-alpha-reductase to the
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48 238 metabolites estradiol and DHT (Schmidt et al. 2008). These sex steroids bind to estrogen (ER)
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50 239 and androgen receptors (AR), respectively, initiating downstream transcriptional effects on
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52 240 peripheral and neural tissues that influence the expression of diverse mating phenotypes
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54 241 (Fuxjager and Schuppe 2018). Sex steroid sensitivity can be evaluated by measuring the
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protein or mRNA abundance of sex steroid receptors and metabolic enzymes, and several aspects of sex steroid sensitivity and metabolism have been measured in SRR species, at least in some peripheral and neural tissues.

Do females and males differ in sensitivity to sex steroids?

The first study to address this question in a SRR system focused on Wilson's phalaropes, in which females showed higher 5-alpha and 5-beta reductase activity in the skin (Schlinger et al. 1989). These differences may explain why females have brighter nuptial plumage than males, but sex differences in neural androgen metabolism did not explain SRR behavior; the sexes did not differ in 5-alpha or 5-beta reductase in the neural tissues sampled. Furthermore, courting male Wilson's phalaropes had higher aromatase activity in the hypothalamus than females, a pattern found in non-SRR species (Balthazart 1991). We are aware of two additional studies that have examined neural sensitivity in SRR species, including black coucals (Voigt and Goymann 2007) and barred buttonquails (Voigt 2016). These studies, like the earlier study in Wilson's phalaropes, focus on the vertebrate social behavior network, an assemblage of steroid-sensitive brain regions that regulate mating, sexual, and social behaviors (Goodson 2005; Maney and Goodson 2011). In black coucals and barred buttonquails, AR mRNA abundance in the nucleus taeniae was higher in females compared to males, suggesting that SRR females may be able to 'do more with less' testosterone in circulation. In species with conventional sex roles, variation in sex steroid sensitivity in the nucleus taeniae may explain variation in aggression, even when hormone levels in the blood do not (Rosvall et al. 2012; Horton et al. 2014). Thus, higher androgen sensitivity in the SRR female nucleus taeniae is an encouraging explanation for SRR. However, not all studies find such patterns. For instance, aromatase gene expression was higher in hypothalamic regions in male barred buttonquails compared to females, a pattern that is comparable to non-SRR Japanese quail (*Coturnix japonica*) (Voigt et al. 2009) and also found in Wilson's phalaropes (Schlinger et al. 1989).

Together, these results suggest modularity of the social behavior network: some aspects of sex steroid sensitivity can be heightened in some neural tissues, and this may vary between females and males.

Global transcriptomic analyses similarly point to sexual dimorphism in sex steroid sensitivity as potentially relevant for SRR species. A microarray study comparing conventional and SRR cichlid species (*Julidodchromis spp*) examined sex differences in gene expression in the whole brain (Schumer et al. 2011). This study found that SRR females had globally similar neural gene expression to *males* in the conventional species, indicating some masculinization of the SRR female brain. Notably, differentially expressed genes between sexes included aromatase and isotocin, a paralog of arginine vasotocin (AVT), which can co-localize in AR+ neurons and influence behavior via steroid sensitive circuits (Kabelik et al. 2010). A recent RNA-seq study of skin and muscle tissue in Gulf pipefish also reported that genes differentially expressed between the sexes have an excess of estrogen response elements, suggesting a role for sex steroids in the genomic regulation of female ornamentation and body depth (Anderson et al. 2020). Thus, similar to non-SRR species (Tomaszycki et al. 2009; Wade 2016), there is potential for sex-biased gene expression to influence sex differences in behavior. Moving forward, these global analyses have the potential to reveal other important mechanisms regulating SRR, particularly if they explicitly link specific nuclei with competitive traits in SRR species and their non-SRR relatives.

What are the ontogenetic origins of sex differences in steroid sensitivity?

Despite good evidence for sex differences in steroid sensitivity in adults, more research is needed to directly link adult data with early life processes in SRR species. To our knowledge, no studies have experimentally manipulated sex steroid exposure early in life to change SRR trait development, and only two studies have investigated the hormonal ontogeny of SRR species. In barred buttonquails, for example, AR mRNA expression levels were higher in female

hatchlings in every brain area investigated, and this sex difference persisted into adulthood for the mediobasal hypothalamus, lateral septum, and nucleus taeniae (Voigt 2016). Notably, these patterns differ from the closely related Japanese quail, a non-SRR species in which AR mRNA abundance is equal in hatchling females and males (Voigt et al. 2009). Another study focused more on testosterone levels in circulation in black coucals, a species that displays the typical SRR pattern in which adult females are larger than males (Andersson 1995). Specifically, Goymann and colleagues (2005) found that female nestlings grow faster and fledge with a larger body mass, compared to males. Although female nestlings do not have higher levels of testosterone in circulation compared to males, structural growth rates are related to testosterone in females but not males (Goymann et al. 2005). Considering connections between androgen exposure and growth, which set the stage for sexual dimorphism in competitive traits in many species (Hews and Moore 1995; Cox et al. 2015), these patterns indirectly suggest organizational effects as plausible drivers of SRR. In non-SRR species, gonadally derived hormones early in development shape neural substrate for activation in adulthood in a sex-specific manner, wherein sexual differentiation results from organizational alignment between gonadal and neural phenotypes (McCarthy 2016). To what extent are gonadal and neural phenotypes mismatched, or more modular, in SRR species? Future work treating embryos and/or juveniles with testosterone or aromatase inhibitors are needed to explicitly test the role of sex steroids in the development of SRR behavior and morphology.

Conclusions and future directions

We evaluated activational and organizational hypotheses linking sex steroids with the development and expression of SRR. Our meta-analysis of sex differences in androgen secretion found that SRR species follow the pattern of conventional species: males have higher levels of androgens in circulation during courtship. Despite stronger selection to compete for mates, SRR females are still females -- they produce ova, solicit copulation, and typically prefer

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3 320 to mate with males. Even at low levels, however, androgens correlate with some competitive
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5 321 phenotypes in SRR females, suggesting that activational effects of androgens may be important
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7 322 in the expression of SRR. These relationships could be reconciled via sex-specific changes in
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9 323 sensitivity to androgens in the neural and peripheral tissues that influence 'reversed' traits in
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11 324 SRR species, such as territorial aggression, plumage coloration, or growth. However, it is still
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13 325 unclear whether and how developmental androgen exposure drives SRR in adults.

14
15 326 With these findings in mind, we see three research initiatives that can move the field
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17 327 towards greater understanding of the role of hormones in the evolution of SRR. First, evidence
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19 328 thus far suggests that tissue-specific sensitivity and organizational effects of androgens may
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21 329 generate SRR, but we need more experiments. Effectively testing these hypotheses will require
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23 330 manipulation of sex steroid levels, metabolism, and/or sensitivity, as well as account for
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25 331 phylogenetic history, for instance using paired designs that directly compare SRR species with
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27 332 non-SRR close relatives. Finding that some SRR phenotypes can be un-reversed, whereas
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29 333 others are fixed, could point to the influence of activational, organizational, or direct genetic
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31 334 effects (Adkins-Regan 2005).

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33 335 Second, we focused on the regulation of competitive traits, but similar hypotheses can
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35 336 apply to the regulation of parental care in SRR males. Although parental care is outside the
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37 337 scope of this review, and historically not part of the definition of SRR (Ah-King and Ahnesjö
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39 338 2013), sex steroids and other hormones like oxytocin, vasopressin, and prolactin are important
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41 339 in the regulation of parental care (Smiley 2019; Storey et al. 2020). In many SRR species,
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43 340 males conduct the majority of parental care and have higher levels of prolactin in circulation
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45 341 than females (Oring et al. 1986, 1988; Gratto-Trevor et al. 1990). In other words, SRR males
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47 342 may have female-typical physiological mechanisms related to parenting. Whether these parental
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49 343 mechanisms are the same that regulate competitive traits (i.e. pleiotropy) or whether these traits
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51 344 are independently regulated in relation to SRR is less clear.

Finally, like many areas of animal behavior, the study of SRR will surely be enhanced by integrated research that explicitly connects ultimate eco-evolutionary processes driving SRR with the proximate neuroendocrine factors that generate trait variation. Ecological feedbacks between the social environment and maternal physiology are a natural area of focus due to potential links between adult trait variation and early life processes. Maternal effects have yet to be tested in SRR species, but evidence from non-SRR species suggests that high-competition environments can influence maternal testosterone allocation to yolk (Bentz et al. 2016). The association of sex-role reversal with male-biased adult sex ratios (Liker et al. 2013) suggests the potential for maternal effects and ecological feedback, as hormones can also influence sex ratios (Navara 2013). This raises the possibility that some physiological regulation of SRR may be environmentally plastic, an exciting arena for future study.

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Figure 1. Mean effect sizes with corresponding 95% confidence intervals for sex differences in levels of androgens in circulation between females and males in courtship or parental stages.

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3371 Black square sizes represent corresponding sampling variances. Grey diamonds represent the

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5372 estimated true effect from the male courtship model ($\mu = -1.14$) and male parenting random-

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7373 effects model ($\mu = -0.019$).

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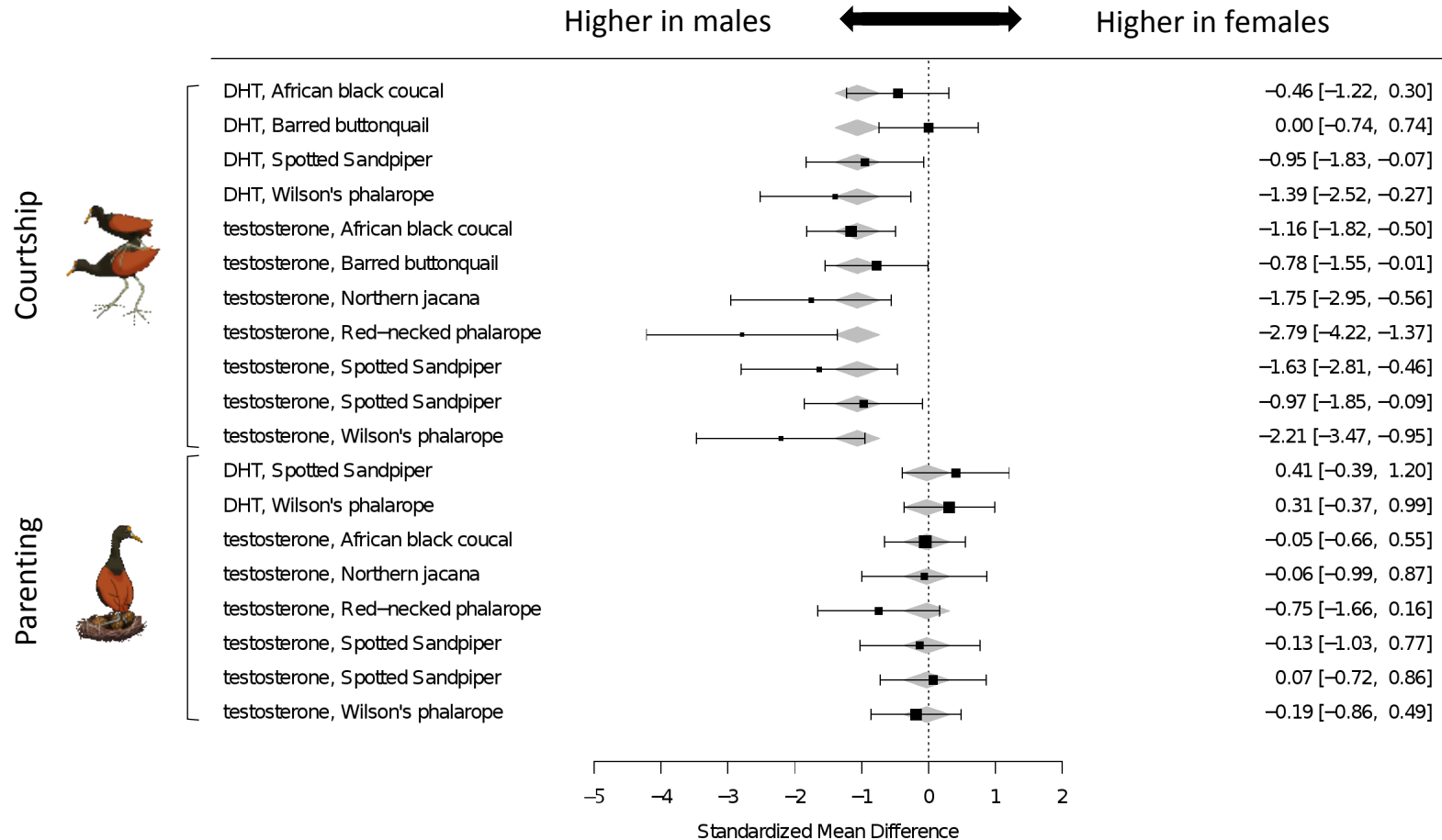
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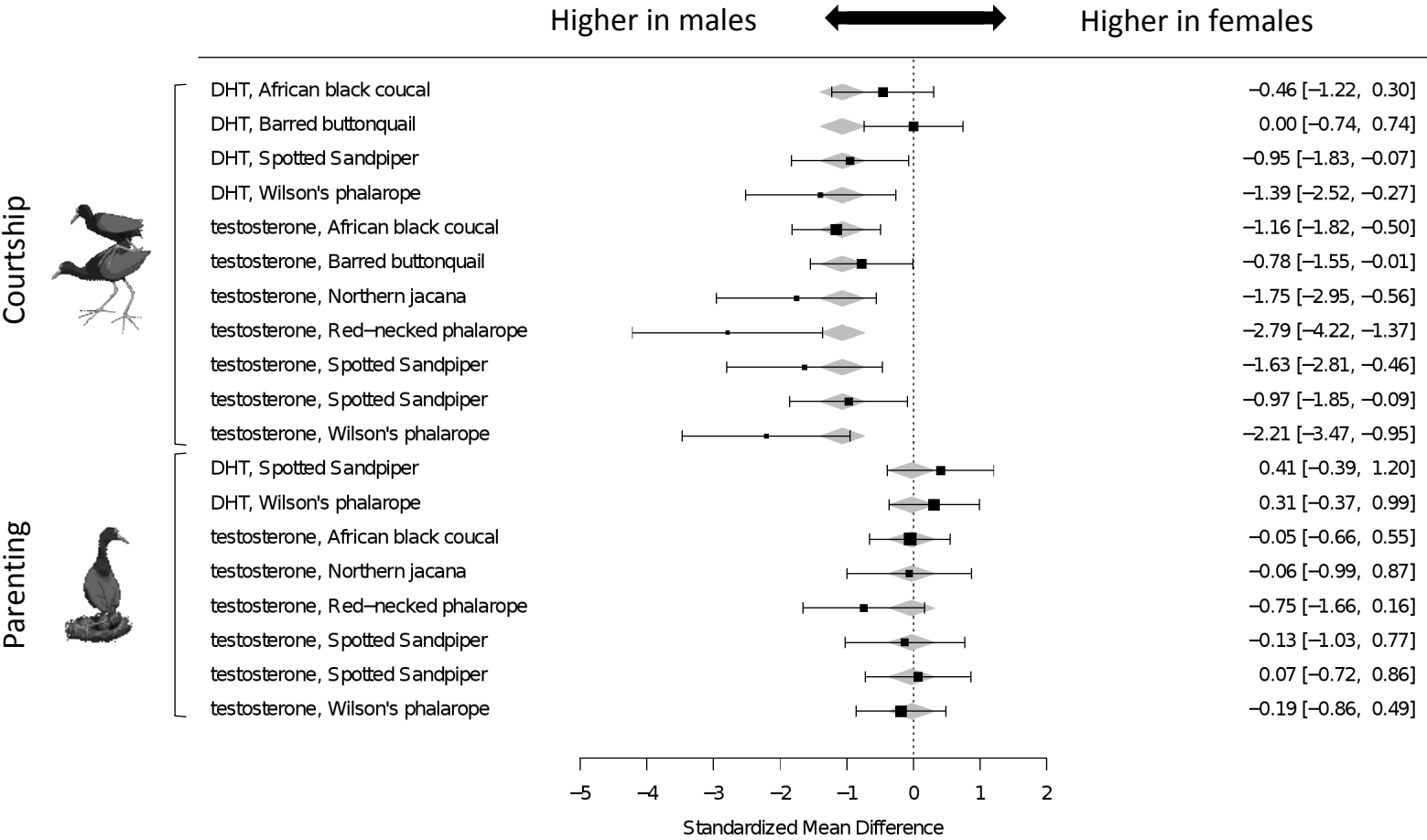
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Trait	Phenotype	Species	Breeding	F_Mean	F_N	F_SD
DHT	Circulating	HoAfrican black c	Courtship	0.095	21	0.119
DHT	Circulating	HoBarred button	Courtship	0.030	14	0.026
DHT	Circulating	HoSpotted Sandp	Courtship	0.120	14	0.075
DHT	Circulating	HoWilson's phalarope	Courtship	0.327	13	0.187
testosterone	Circulating	HoAfrican black c	Courtship	0.567	25	0.295
testosterone	Circulating	HoBarred button	Courtship	0.200	14	0.374
testosterone	Circulating	HoNorthern jaca	Courtship	0.510	12	0.450
testosterone	Circulating	HoRed-necked phalarope	Courtship	0.085	8	0.133
testosterone	Circulating	HoSpotted Sandp	Courtship	0.630	8	0.651
testosterone	Circulating	HoSpotted Sandp	Courtship	0.180	14	0.112
testosterone	Circulating	HoWilson's phalarope	Courtship	0.505	13	0.343
DHT	Circulating	HoSpotted Sandp	Parental	0.120	14	0.075
DHT	Circulating	HoWilson's phalarope	Parental	0.327	13	0.187
testosterone	Circulating	HoAfrican black c	Parental	0.567	25	0.295
testosterone	Circulating	HoNorthern jaca	Parental	0.510	12	0.450
testosterone	Circulating	HoRed-necked phalarope	Parental	0.085	8	0.133
testosterone	Circulating	HoSpotted Sandp	Parental	0.630	8	0.651
testosterone	Circulating	HoSpotted Sandp	Parental	0.180	14	0.112
testosterone	Circulating	HoWilson's phalarope	Parental	0.505	13	0.343

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M_Mean	M_N	M_SD	Citation	Notes
0.179		10	0.266 Voigt and Goy	Values came from webplot digitizer
0.030		14	0.030 Voigt 2016	
0.200		9	0.090 Rissman and Wingfield 1984	
0.907		5	0.722 Fivizzani et al. 1986	
2.157		17	2.095 Goymann and	Values came from hormonebase
0.500		14	0.374 Voigt 2016	
5.260		5	4.919 Lipshutz and Rosvall	
3.979		7	1.926 Gratto-Trevor combined data from all 2 years	
3.710		7	2.513 Oring et al. 19	Values came from hormonebase
0.950		9	1.230 Rissman and Wingfield 1984	
3.608		5	2.609 Fivizzani et al. 1986	
0.090		11	0.066 Rissman and Wingfield 1984	
0.262		24	0.216 Fivizzani et al. 1986	
0.590		18	0.573 Goymann and Wingfield 2004	
0.540		7	0.476 Lipshutz and Rosvall	
0.222		13	0.198 Gratto-Trevor et al. 1990 GCE	
0.740		12	0.901 Oring et al. 1986	
0.170		11	0.166 Rissman and Wingfield 1984	
0.625		24	0.740 Fivizzani et al. 1986	