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SYMPOSIUM

Neuroendocrinology of Sex-Role Reversal

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Synopsis 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 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 effects of androgens may be sex-specific; circulating androgen levels correlate with some competitive phenotypes in sex-role reversed females. We also review evidence that sex-role reversed females have higher tissue-specific sensitivity to androgens than males, at least in some species and tissues. Organizational effects may explain these relationships, considering that early exposure to sex steroids can shape later sensitivity to hormones, often in sex-specific ways. Moving forward, experimental and correlative studies on the ontogeny and expression of sex-role reversal will further clarify the mechanisms that generate sex-specific behaviors and sex roles.

AQ2

> "Why are males masculine, females feminine and occasionally vice-versa?"
>
> – GC Williams, 1975

An introduction to sex-role reversal

Why are females and males different? Biologists have been trying to answer this question since Darwin first postulated that "instinct" (behavior) may be shaped by natural selection (1859). In his Victorian era, male animals were considered dominant and promiscuous, whereas female animals were thought to be coy and subdued (Hrdy 1986). In the intervening years, we have learned a lot about "sex roles" across the animal kingdom. For many species, as Darwin and his contemporaries observed males face stronger competition for mating opportunities than females, and females tend to conduct the majority of parental care (Darwin 1871; Clutton-Brock 1991; Andersson 1994). Whereas territorial aggression and promiscuity were historically considered male traits, behavioral ecologists now recognize that intrasexual competition and multiple-mating are adaptive and widespread behaviors in females of many species (Clutton-Brock 2009; Rosvall 2011; Hare and Simmons 2019). Sex-role reversal occurs when sexual selection among females is stronger than sexual selection acting among males, and it is typically (but not necessarily) associated with polyandry, male mate choice, and male-only parental care (Vincent et al. 1992; Kvarnemo and Ahnesjo 1996; Ah-King and Ahnesjö 2013). Sex-role reversed females are characterized by phenotypes typically associated with males, including morphological traits like heavier body mass, larger weaponry, and more ornamentation, as well as behavioral traits like higher territorial aggression or more intense courtship rituals. These traits are thought to facilitate female–female competition for mates and breeding territories (Emlen and Oring 1977; Gwynne 1991), though sexual size dimorphism may also relate to fecundity and/or viability selection (Blanckenhorn 2005; Pincheira-Donoso and Hunt 2017). While we focus on sex-role reversal here, it is important to note that

sexual selection acts on females in many species with "conventional" sex roles (Rosvall 2011; Hare and Simmons 2019), and that categorizing sex roles as "conventional" versus "reversed" oversimplifies the complexity of behavioral diversity (Gowaty 2004; Roughgarden 2004; Ah-King and Ahnesjö 2013; Orr et al. 2020, this edition).

Classic work by Bateman (1948) and Trivers (1972) attributed the evolution of sex roles to anisogamy, although this has proven challenging to reconcile with sex-role reversal. In general, male gametes (sperm) are smaller and more numerous than nutrient-rich female gametes (ova), and so males may be more available to mate than females, who may be predisposed to caring for their offspring based on this initial asymmetry in parental investment. For decades, this anisogamy argument dominated theory on the evolution of sex roles: sex differences in initial gametic investment drive the degree of mating competition and direction of sexual selection (reviewed in Tang-Martinez 2016; Hoquet 2020). More recent theoretical work suggests that sex-specificity of sexual selection is also driven by multiple paternity, adult mortality, and sex ratios (Kokko and Jennions 2008; Fromhage and Jennions 2016). The broader debate on anisogamy and sex-role evolution is interesting to consider in light of sex-role reversal, because sex-role-reversed females are nonetheless female; they always produce the larger gamete, even when they compete for mates more than males compete for mates. Several different ecological factors are thought to set the stage for sex role reversal, including sex ratio, availability of mates, and distribution of resources (Emlen and Oring 1977; Gwynne 1991; Andersson 2005; Liker et al. 2013; Fritzsche et al. 2016).

Much less is known about the proximate mechanisms that underlie sex-role reversal. Sex steroids are logical candidates for the physiological regulation of sex-role reversal, because these hormones are associated with many sexually dimorphic traits, especially those related to mating and mating competition (Adkins-Regan 2005). The actions of sex steroids are generally classified in two main ways, the first of which operates early in life during a critical period when exposure to a hormone (or lack thereof) can permanently organize tissue structure and function that is, organizational effects (Phoenix et al. 1959; Arnold and Breedlove 1985), which determine whether later exposure to a hormone can bring about a phenotypic effect. One of the primary modes of action for organizational effects is to change the anatomical distribution and/or abundance of sex steroid receptors, early in life and often lasting into adulthood (Moore et al. 1998). Such changes in sensitivity can also occur on shorter time scales in adulthood, for instance, in response to a social challenge (Fuxjager et al. 2010). Activational effects typically occur during adulthood, when animals change aspects of their phenotype in response to changing hormone levels in circulation. For sex steroids, hormone secretion is regulated by the hypothalamic–gonadal–pituitary axis, when external stimuli prompt the hypothalamus to secrete gonadotropin-releasing hormone (GnRH). GnRH then stimulates the pituitary to release gonadotropins luteinizing hormone and follicle-stimulating hormone into the bloodstream. These gonadotropins signal to the gonads to initiate gametogenesis as well as produce sex steroids including estrogens, progestogens, and androgens (Schulz et al. 2010). Many researchers have found important connections between androgens, 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 androgenic signaling may contribute to the origin and expression of sex-role reversal.

Here, we evaluate four key questions on the role of sex steroids in the regulation of sex-role reversal and associated phenotypes. We begin by evaluating evidence that sex-role reversal may be explained by sex differences in sex steroid signaling, focusing on (1) levels of androgens in circulation, as well as (2) co-variation between androgens and competitive phenotypes. Next, we ask whether (3) adult sex differences in tissue-level sensitivity to hormones (e.g., androgen receptor abundance) may account for sex-role reversal, and finally, we explore (4) the ontogenetic origins of sex-role reversal, focused on organizational effects of androgens. We highlight examples from birds and fishes, which have received the most attention to date in the study of sex-role reversal, and we draw inferences from species with conventional sex roles.

Do sex-role reversed females and males differ in androgen levels in circulation?

Two decades ago, Eens and Pinxten (2000) contributed an important review on the behavior and endocrinology of sex-role reversed species. They evaluated the hypothesis that sex-role reversed females have male-typical physiological mechanisms,

specifically that testosterone secretion may be higher in sex-role reversed females, a reversal from the conventional pattern. Evidence from three sex-role reversed 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 testosterone in sex-role reversed species. For the six sex-role reversed avian species with relevant data, we compiled mean testosterone 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 S1). Our analysis, therefore, updates Eens and Pinxten's findings with more recent studies on testosterone secretion in sex-role reversed avian species including black coucals (*Centropus grillii*), barred buttonquails (*Turnix suscitator*), and northern jacanas (*Jacana spinosa*) (Goymann and Wingfield 2004; Voigt and Goymann 2007; Muck and Goymann 2011; Voigt 2016; Lipshutz and Rosvall 2020). The majority of these studies were conducted on free-living individuals in their natural environments, except for barred buttonquails, for which hormonal data have only been measured in captivity. To estimate the standardized effect size of sex differences in circulating testosterone across sex-role reversed species, we used random effects models in the package metafor in R (Viechtbauer 2010). We compare species using sex difference summaries, rather than mean levels, because these studies used different methods, which can inflate variation based on technical approach rather than biological reality (Goymann and Wingfield 2014; Fanson et al. 2017). We ran separate models comparing females to males in different breeding stages (i.e., courting versus caring), as male androgen levels typically decline with parental care (Wingfield et al. 1990) and role-reversed females do not conduct parental care. 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 testosterone in circulation.

First, focused on testosterone data from females and males, sampled when both sexes were competing and courting, our analysis showed an average negative and significant effect size ($\mu = -1.30$, $z = -5.97$, $P < 0.0001$), indicating that sex-role reversed males have higher testosterone in circulation than females (Fig. 1). Thus, during the period of time when both sexes are seeking mates, sex-role reversed males secrete higher levels testosterone than females, which follows patterns found in species with conventional sex roles (Goymann and Wingfield 2014). These patterns support the findings of Eens and Pinxten (2000).

When we analyzed testosterone levels in sex-role reversed birds during the period of time when males are parenting, however, the effect size was not significant ($\mu = -0.155$, $z = -0.967$, $P = 0.33$), indicating no overall sex difference in circulating testosterone levels (Fig. 1). During the parental care phase of breeding, these sex-role reversed males and females did not differ significantly in levels of testosterone in circulation, meaning that these hormonal differences between the sexes essentially disappear during periods of male parental care. Indeed, direct comparison of the courtship- and parental-stage data into a single model shows that breeding stage explained 100% of the variation in effect size ($Q_M = 21.11$, df = 1, $P < 0.0001$). We find it interesting that in sex-role reversed species, sex differences in circulating androgen levels are most reduced during a breeding stage when the sexes are most behaviorally divergent, that is, males are conducting parental care, but females are still competing for mating opportunities with other males. Clearly, sex differences in levels of testosterone in circulation alone do not explain sex-role reversal.

These patterns suggest that sex-role reversal may drive lower testosterone levels in males, higher testosterone levels in females, or some combination of the two (sensu Goymann and Wingfield 2014). In species with conventional sex roles, testosterone levels in both females and males decline during parental care (Wingfield et al. 1990; Ketterson et al. 2005; Hirschenhauser and Oliveira 2006), which may also reduce the degree of sexual dimorphism. Direct phylogenetic comparison to species with conventional sex roles is needed to evaluate whether the erasure of sexual dimorphism in circulating testosterone is typical or unique to sex-role reversed species. Data are currently limited across avian families except for *Scolopacidae*, which includes phalaropes and sandpipers. During parental care, sex-role reversed male Red-necked phalaropes have 2.6× higher testosterone in circulation than females (Gratto-Trevor et al. 1990), male Wilson's phalaropes have 1.2× (Fivizzani et al. 1986), and male spotted sandpipers range from 0.94 to 1.2× (Rissman and Wingfield 1984; Fivizzani et al. 1986), but none of these levels

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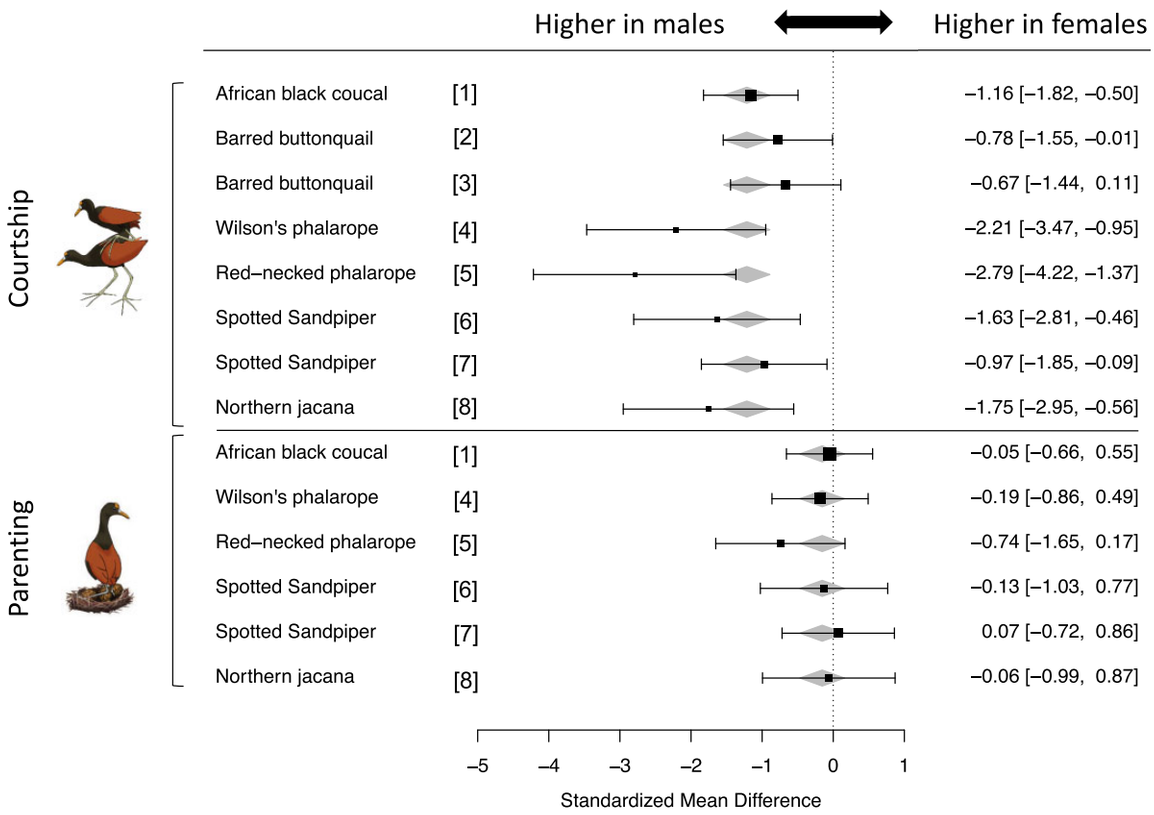

Fig. 1 Mean effect sizes with corresponding 95% confidence intervals for sex differences in levels of testosterone in circulation between females and males in courtship or parental stages. Black square sizes represent corresponding sampling variances. Gray diamonds represent the estimated true effect from the male courtship model ($\mu = -1.30$) and male parenting random-effects model ($\mu = -0.155$). Numbers refer to references: [1] Goymann and Wingfield 2004, [2] Voigt 2016, [3] Muck and Goymann 2011, [4] Fivizzani et al. 1986, [5] Gratto-Trevor et al. 1990, [6] Oring et al. 1986, [7] Rissman and Wingfield 1984, [8] Lipshutz and Rosvall 2020. All studies were conducted on free-living individuals in their natural environments, except for barred buttonquails [2, 3].

AQ10

differ significantly between the sexes. Semipalmated sandpipers (*Calidris pusilla*) have biparental care and conventional sex roles, but males have 2–5× greater testosterone in circulation than females during incubation and brooding (Gratto-Trevor et al. 1990; Steiger et al. 2006). Conventional-role pectoral sandpipers (*Calidris melanotos*) have female-only parental care, and testosterone levels were 34× higher in males than in females during the parental care stage (Steiger et al. 2006). These comparisons within *Scolopacidae* support our expectation that sex differences in testosterone are erased during parental care for sex-role reversed species, but not for those with conventional sex roles.

Our finding that sex differences in testosterone are greater during courtship than parental care in sex-role reversed species also appears to apply to other androgens, including testosterone's more potent metabolite, 5-alpha dihydrotestosterone (DHT) (Rissman and Wingfield 1984; Fivizzani et al. 1986; Voigt and Goymann 2007; Voigt 2016). Levels of DHT and testosterone within a species are often correlated in both sexes (Fivizzani et al. 1986; Goymann et al. 2001; Nowak et al. 2018), though not always in females (Steiger et al. 2006). An androgen found in fishes, 11-ketotestosterone, is higher in males than females in sex-role reversed broadnosed pipefish (*Syngnathus typhle*) and greater pipefish (*Syngnathus acus*), though this sex difference decreases as males shift from courting to brooding (Mayer et al. 1993). This decreasing in androgens during the parental stage is also reflected in nonsex-role-reversed teleost fishes (Knapp et al. 1999; Mayer et al. 2004; Scobell and Mackenzie 2011).

Beyond androgens, fewer studies have measured other sex steroids or prohormones in sex-role reversed 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 androstenedione and dehydroepiandrosterone is similar between the sexes,

regardless of breeding stage (Goymann and Wingfield 2004). Estradiol levels may be higher in females than males in some sex-role reversed species (Fivizzani et al. 1986), or higher than expected in sex-role reversed males (Rissman and Wingfield 1984; Goymann and Wingfield 2004; Voigt 2016). This mixed evidence for sex differences in estradiol is perhaps related to the low, sometimes undetectable concentrations in both sexes (e.g., Rosner et al. 2013; Voigt 2016), which may relate to the difficulty of capturing peak levels in the sampling regime. Progesterone levels are also typically higher in females than males, which is reflected in some sex-role reversed species (Fivizzani et al. 1986; Gratto-Trevor et al. 1990), but not others (Voigt 2016). Together, these studies suggest that the sex steroid profiles of sex-role reversed females and males are similar to their counterparts with conventional sex roles.

Are activational effects of androgens sexually dimorphic in relation to sex-role reversal?

There is good evidence that androgens can have opposing effects on sexually dimorphic phenotypes, potentially driven by sex differences in gene regulatory responses to sex steroids (Van Nas et al. 2009; Peterson et al. 2014). The effects of androgens on sexually dimorphic phenotypes may also differ among species. For instance, in squamate reptiles, testosterone inhibits growth in species with female-biased sexual size dimorphism, and testosterone stimulates growth in species with male-biased sexual size dimorphism (Cox et al. 2009). Although this example relates to developmental processes, it illustrates how androgens have the potential to differentially affect the sexes. Thus, females and males in sex-role reversed species might differ in the activational effects of androgens, 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 (1) correlations that directly link sex-role reversed behaviors and morphological traits with hormone levels, or more directly (2) experimental treatment with exogenous androgens.

One approach to understand the physiological regulation of sex-role reversal 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 sex-role reversed 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 sex-role reversed females, there is some evidence that testosterone is related to the regulation of competitive traits in sex-role reversed females in some way. Other studies find that nonsteroid hormones may regulate competitive traits in relation to sex-role reversal. In the two-spotted goby (*Gobiusculus flavescens*), a species with dynamic sex roles that change from conventional to reversed (Forsgren et al. 2004), females have an orange belly ornament used in both courtship and intersexual competition, and this ornament is absent from males (Amundsen and Forsgren 2001). Pigmentation of the female belly ornament is regulated by the pituitary hormone prolactin as well as alpha-melanocyte stimulating hormone, but this female 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 sex-role reversed species are 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 sex-role reversed 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 a synthetic 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 (Lank et al. 1999; Lindsay et al. 2016). In other words, the activational effects of sex steroids can reverse sex-specific phenotypes in sex-role reversed males in a manner similar to conventional-role females. For competitive behaviors like aggression, the link with testosterone has received mixed support, both in sex-role reversed species and those with conventional sex roles (Rosvall et al. 2019; Wingfield

et al. 2019). In female barred buttonquails, implantation with testosterone did not increase aggression, and territorial challenge decreased levels of testosterone in circulation (Muck and Goymann 2019). In female black coucals, testosterone did not differ between challenged and unchallenged females, but territorial challenge decreased levels of progesterone in circulation, and progesterone implants reduced female aggression (Goymann et al. 2008). One possibility is that behaviors under androgenic control in males of conventional-role species may be regulated by other hormones in females of sex-role reversed species, similar to the change from androgenic to progestogenic regulation of mounting behavior in whiptail lizards (Crews 2005). In sum, variation in androgens in circulation may explain some competitive phenotypes but not others, and these relationships can vary by sex, suggesting we must also look for alternative explanations for the physiological origin and expression of sex-role reversal.

Do sex-role reversed females and males differ in sensitivity to sex steroids?

Androgen levels themselves are only part of the regulation of androgen-mediated phenotypes, which also includes mechanisms of sex steroid sensitivity (Fuxjager and Schuppe 2018). Sensitivity comprised a number of factors, including sex steroid receptors, as well as enzymes that produce steroid hormones and convert them into more or less active forms (Ball and Balthazart 2008). In particular, testosterone can be locally converted by the enzymes aromatase and 5-alpha-reductase to the metabolites estradiol and DHT (Schmidt et al. 2008). These sex steroids bind to estrogen and androgen receptors (ER and AR), respectively, initiating downstream transcriptional effects on peripheral and neural tissues that influence the expression of diverse mating phenotypes (Fuxjager and Schuppe 2018). Sex steroid sensitivity can be evaluated by measuring the protein or mRNA abundance of sex steroid receptors and metabolic enzymes, and several aspects of sex steroid sensitivity and metabolism have been measured in sex-role reversed species, at least in some tissues.

The first study to address whether sex-role reversed females and males differ in sensitivity to sex steroids 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 sex-role reversed behavior; the sexes did not differ in 5-alpha or 5-beta reductase in the brain regions sampled. Furthermore, courting male Wilson's phalaropes had higher aromatase activity in the hypothalamus than females, a pattern found in nonsex-role reversed species (Balthazart 1991). We are aware of two additional studies that have examined neural sensitivity in sex-role reversed 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 adult females compared to males, suggesting that sex-role reversed 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 sex-role reversed female nucleus taeniae is an encouraging explanation for sex-role reversal. 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 nonsex-role reversed 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 sex-role reversal. A microarray study comparing conventional and sex-role reversed cichlid species (*Julidodchromis spp)* examined sex differences in gene expression in the whole brain (Schumer et al. 2011). This study found that sex-role reversed females had globally similar neural gene expression to males in the conventional species, indicating some masculinization of the sex-role reversed female brain. Notably, differentially expressed genes between sexes included aromatase and isotocin, a paralog of arginine vasotocin, 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 nonsex-role reversed 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 sex-role reversal, particularly if they explicitly link specific nuclei with competitive traits in sex-role reversed species and their nonsex-role reversed relatives.

To what degree do organizational effects of sex steroids influence sex-role reversal?

Organizational effects of sex steroids deliver some promise as proximate regulators of sex-role reversal (Adkins-Regan 2012). An ontogenetic hypothesis for sex-role reversal was proposed by Fivizzani et al. (1986), drawing from observations in many species that exposure to sex steroid hormones early in development can generate sex differences in later responsiveness to these hormones in adulthood (Arnold 2009). For instance, exposure to high testosterone early in life, *in utero*, or *in ovo* can influence suites of sexual characteristics (vom Saal and Bronson 1980; Hotchkiss et al. 2007), a phenotypic effect that is at least partly mediated by tissue level changes in sensitivity to sex steroids (Mori et al. 2010; Pfannkuche et al. 2011), as well as other changes to anatomy, neuronal growth, and circuitry.

To our knowledge, no studies have experimentally manipulated sex steroid exposure early in life to change trait development in a sex-role reversed species. However, two studies have investigated the ontogeny of hormonal signaling sex-role reversed species. Goymann et al. (2005) compared sex differences in testosterone levels in nestling black coucals, a species for which adult females are larger than males (Andersson 1995), and nestlings are altricial and receive extensive male care (Goymann et al. 2016). Female nestlings grew faster and fledged with a larger body mass than males, but females had lower levels of testosterone in circulation. However, structural growth rates were related to testosterone in females, but not in males, suggesting a role for sex-specific sensitivity (Goymann et al. 2005). Considering connections between androgen exposure and growth, which influence sexual dimorphism in competitive traits for many species (Hews and Moore 1995; Cox et al. 2015), these patterns point to organizational effects as plausible drivers of sex-role reversal. In the other study, Voigt (2016), compared sex differences in steroid receptors in hypothalamic and limbic brain regions in hatchling barred buttonquails. Female hatchlings had higher AR mRNA expression levels 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 Japanese quail, a nonsex-role reversed species in which AR mRNA abundance is equal in hatchling females and males (Voigt et al. 2009). As of yet, it is unknown whether barred buttonquail sex differences in gene expression arose from hormonal organization early in development, as levels of estradiol, DHT, testosterone, and progesterone were either below the detection limit or did not significantly differ between females and males.

In species with conventional sex roles, gonadally derived hormones early in development can shape neural substrates for activation in adulthood in a sex-specific manner, wherein sexual differentiation results from organizational alignment between gonadal and neural phenotypes (McCarthy 2016). Future work treating embryos and/or juveniles with testosterone or aromatase inhibitors is needed to explicitly test the role of sex steroids in the development of sex-role reversed behavior and morphology, and we also welcome more observational studies on the ontogeny of sex steroid signaling. These effects need not be plastic, and indeed chromosomal inversions can change hormone signaling and suites of sexually dimorphic traits in ways that may facilitate sex-role reversal (Horton et al. 2014; Küpper et al. 2016).

Conclusions and future directions

We evaluated four nonmutually exclusive pathways linking sex steroids with the development and expression of sex-role reversal. Our meta-analysis of sex differences in testosterone secretion found that sex-role reversed species follow the pattern of conventional species: males have higher levels of androgens in circulation during courtship. Despite stronger selection to compete for mates, sex-role reversed females are still females—they produce ova, solicit copulation, and typically prefer to mate with males. Even at low levels, however, androgens correlate with some competitive phenotypes in sex-role reversed females, suggesting that activational effects of androgens may be important in the expression of sex-role reversal. These relationships could be reconciled via sex-specific changes in sensitivity to

androgens in the neural and peripheral tissues that influence "reversed" traits in sex-role reversed species, such as territorial aggression, plumage coloration, or growth. However, it is still unclear whether and how developmental androgen exposure drives sex-role reversal in adults.

With these findings in mind, we see three research initiatives that can move the field toward greater understanding of the role of hormones in the evolution of sex-role reversal. First, evidence thus far suggests that tissue-specific sensitivity and organizational effects of androgens have the potential to generate sex-role reversal, but we need more experiments. Effectively testing these hypotheses will require manipulation of sex steroid levels, metabolism, and/or sensitivity, as well as account for phylogenetic history, for instance using paired designs that directly compare sex-role reversed species with nonsex-role reversed close relatives. Findings that some sex-role reversed phenotypes can be un-reversed, whereas others are fixed, could point to the influence of activational, organizational, or direct genetic effects (Adkins-Regan 2005).

Second, we focused on the regulation of competitive traits, but similar hypotheses can apply to the regulation of parental care in sex-role reversed males. Although parental care is outside the scope of this review, sex steroids and other hormones like oxytocin, vasopressin, and prolactin are important in the regulation of parental care (Smiley 2019; Storey et al. 2020). In many sex-role reversed species, including ones featured here, males conduct the majority of parental care and have higher levels of prolactin in circulation than females (Oring et al. 1986, 1988; Gratto-Trevor et al. 1990). Whether these parental mechanisms are the same that regulate competitive traits (i.e., pleiotropy) or whether these traits are independently regulated in relation to sex-role reversal is less clear.

Finally, like many areas of animal behavior, the study of sex-role reversal will surely be enhanced by integrated research that explicitly connects eco-evolutionary processes driving sex-role reversal with the proximate 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 sex-role reversed species, but evidence from nonsex-role reversed species suggests that high-competition environments can influence maternal testosterone allocation to yolk, at least in some species (Bentz et al. 2016). The potential for ecological and social feedback raises the possibility that some physiological regulation of sex-role reversal may be environmentally plastic, an exciting arena for future study.

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AQ3
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Supplementary data

Supplementary data available at *ICB* online.

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