Effects of Testosterone on Gene Expression Are Concordant between Sexes but Divergent across Species of *Sceloporus* Lizards

Christopher D. Robinson,[1,*] Matthew D. Hale,[1,2] Christian L. Cox,[3] Henry B. John-Alder,[4] and Robert M. Cox[1]

1. Department of Biology, University of Virginia, Charlottesville, Virginia, 22903; 2. US Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, Maryland 20910; and Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, Maryland 20817; 3. Department of Biological Sciences and Institute of Environment, Florida International University, Miami, Florida 33181; 4. Department of Ecology, Evolution, and Natural Resources, Rutgers University, New Brunswick, New Jersey 08901

Submitted March 29, 2024; Accepted June 28, 2024; Electronically published September 24, 2024

Online enhancements: supplemental PDF.

ABSTRACT: Hormones mediate sexual dimorphism by regulating sex-specific patterns of gene expression, but it is unclear how much of this regulation involves sex-specific hormone levels versus sex-specific transcriptomic responses to the same hormonal signal. Moreover, transcriptomic responses to hormones can evolve, but the extent to which hormonal pleiotropy in gene regulation is conserved across closely related species is not well understood. We addressed these issues by elevating testosterone levels in juvenile females and males of three *Sceloporus* lizard species before sexual divergence in circulating testosterone and then characterizing transcriptomic responses in the liver. In each species, more genes were responsive to testosterone in males than in females, suggesting that early developmental processes prime sex-specific transcriptomic responses to testosterone later in life. However, overall transcriptomic responses to testosterone were concordant between sexes, with no genes exhibiting sex-by-treatment interactions. By contrast, hundreds of genes exhibited species-by-treatment interactions, particularly when comparing distantly related species with different patterns of sexual dimorphism, suggesting evolutionary lability in gene regulation by testosterone. Collectively, our results indicate that early organizational effects may lead to sex-specific differences in the magnitude, but not the direction, of transcriptomic responses to testosterone and that the hormone-genome interface accrues regulatory changes over evolutionary time.

Keywords: evolutionary endocrinology, evolutionary potential hypothesis, organizational/activational hypothesis, pleiotropy, sexual dimorphism.

* Corresponding author; email: cdr4ua@virginia.edu.

ORCIDs: Robinson, https://orcid.org/0000-0002-1874-4660; Hale, https://orcid.org/0000-0003-0338-847X; C. L. Cox, https://orcid.org/0000-0002-9424-8482; John-Alder, https://orcid.org/0000-0001-5036-592X; R. M. Cox, https://orcid.org/0000-0001-8325-111X.

Introduction

Hormones with sex-specific patterns of secretion, such as androgens and estrogens, mediate the development of sexually dimorphic phenotypes by facilitating the sex-specific transcription of a shared autosomal genome (Rinn and Snyder 2005; van Nas et al. 2009; Partridge et al. 2015; Cox et al. 2017; Anderson et al. 2020; Oliva et al. 2020; Hale et al. 2022). Two questions about this hormonal regulation of gene expression are key to understanding the evolutionary dynamics of sexual dimorphism. First, are sex differences in gene expression achieved primarily through sexual divergence in hormone levels during maturation, or do the sexes also differ in their transcriptomic responses to the same hormonal signal? Second, to what extent are the regulatory effects of hormones evolutionarily labile across species? The first question about the sex-specificity of hormonal regulation touches on the classic endocrine paradigm of sex-specific organizational effects of hormones that occur early in development and thereby shape responsiveness to activational effects of elevated hormone levels later in life (Phoenix et al. 1959; Arnold and Breedlove 1985; Arnold 2009; McCarthy et al. 2009; Adkins-Regan 2012; Madison et al. 2015; McCarthy 2016; Anderson et al. 2022). The second question about the species specificity of hormonal regulation is central to current debate over whether the regulatory architecture of hormonal pleiotropy acts primarily as an evolutionary constraint or instead represents an adaptable source of evolutionary potential (Ketterson and Nolan 1999; Hau 2007; McGlothlin and Ketterson 2008; Cox et al. 2009; Ketterson et al. 2009; Hau and Wingfield

  https://doi.org/10.1086/732200

2011; Lema 2014; Fuxjager and Schuppe 2018; Cox 2020). In this study, we address both questions by simultaneously characterizing the sex and species specificity of hormonally mediated gene expression in the lizard genus *Sceloporus*, in which the hormone testosterone is known to mediate many phenotypic sexual dimorphisms (Quinn and Hews 2003; Cox et al. 2005*a*, 2005*b*, 2008) and underlying patterns of gene expression (Robinson et al. 2023).

In many sexually dimorphic species, exogenous testosterone is sufficient to induce male-typical phenotypes in females (Tobias et al. 1991; Rhen et al. 1999; Cox et al. 2005*a*. 2015; Lahaye et al. 2012, 2014; Lindsay et al. 2016; Rose et al. 2022). In some of these cases, testosterone has also been shown to induce similar patterns of genetic covariance and gene expression in each sex (Cox et al. 2017; Wittman et al. 2021). Although only a few studies have directly compared transcriptome-wide responses to testosterone between the sexes, these studies have revealed relatively little overlap in the specific genes identified as differentially expressed in each sex (Peterson et al. 2013, 2014; Hale et al. 2022). This apparent sex specificity could occur because early developmental exposure to androgens, estrogens, and other factors can mediate the strength of subsequent hormonal responsiveness by altering the availability of hormone receptors, transcriptional cofactors, or enzymes for hormone metabolism (McAbee and DonCarlos 1998; Bodo and Rissman 2008; Manoli and Tollkuhn 2018; Neubert da Silva et al. 2019; Gegenhuber and Tollkuhn 2020; Lagunas et al. 2022). Therefore, early organizational effects can predispose females and males to different transcriptional responses to hormones later in life (Fiber and Swann 1996; Sullivan et al. 2009; Chinnathambi et al. 2013; Peterson et al. 2013, 2014; Schweitzer et al. 2013), limiting the phenotypic space available to each sex (Dufty et al. 2002; Adkins-Regan 2007). Studies investigating organizational effects of hormones during embryonic growth have focused on neural development and the subsequent activation of adult reproductive behaviors (Phoenix et al. 1959; McCarthy et al. 2009; McCarthy 2016), although evidence of organization has been observed in other tissues and for other phenotypes (Hews and Moore 1995; Rosa-Molniar et al. 1996; Arnold 2009). Here, we explore whether this framework of sex-specific organization and activation can be extended to other tissues and to gene regulatory processes by testing for sex-specific transcriptomic responses to hormone treatments that simulate activational levels of testosterone typical of adult males.

Phenotypic responses to hormones can also differ between closely related populations or species (Kitano et al. 2011; Bergeon Burns et al. 2014; Frankl-Vilches et al. 2015; Rosvall et al. 2016*a*, 2016*b*; Cox et al. 2022*a*; Robinson et al. 2023). However, the evolution of transcriptional responses to hormones and the constraints imposed by pleiotropic gene regulation by the same hormone (i.e., hormonal pleiotropy) are poorly understood (Fuxjager et al. 2018; Cox 2020; Cox et al. 2022*b*; Rosvall 2022; Anderson and Renn 2023; Davidson et al. 2023). Comparing this regulatory architecture across related species can help assess the evolutionary lability of hormone-gene couplings that underlie hormonal pleiotropy (Cox et al. 2022*b*). This evolutionary lability is important because fitness trade-offs can arise when selection acts on multiple phenotypes regulated by the same hormone (Stearns 1989; Flatt et al. 2005; Hau 2007; Roff and Fairbairn 2007; Mauro and Ghalambor 2020), causing shifts away from fitness peaks for some traits if the regulatory effects of hormones are evolutionarily conserved (McGlothlin and Ketterson 2016; Dantzer and Swanson 2017; Wittman et al. 2021; Cox et al. 2022*b*). This view of hormonal pleiotropy is known as the evolutionary constraint hypothesis (Hau 2007). In contrast, the evolutionary potential hypothesis (Hau 2007) proposes that couplings between hormones and the downstream phenotypes they regulate are evolutionarily labile, thereby facilitating adaptation (McGlothlin and Ketterson 2008). For example, the evolution of testosterone-mediated phenotypes, such as foot-flagging behavior in frogs (Mangiamele et al. 2016; Mangiamele and Fuxjager 2018; Anderson et al. 2021), wing-snap displays in manakin birds (Fuxjager et al. 2015), and locomotor and push-up behaviors in *Anolis* lizards (Johnson et al. 2018), result from the evolution of tissue-specific abundance of androgen receptors. Evolutionary changes in coregulator recruitment and local hormone conversion can also facilitate evolutionary changes in the hormonal sensitivity of entire tissues or cell types (Fuxjager and Schuppe 2018; Cox et al. 2022*b*), but much less is known about the evolution of hormonal responsiveness for individual genes and pathways within these tissues and cells. Transcriptomes provide data-rich descriptions of the pleiotropic regulatory effects of hormones (Peterson et al. 2013; Kitano et al. 2014; Peterson et al. 2014; Fuxjager et al. 2016; Cox et al. 2017; Finseth and Harrison 2018; Newhouse and Vernasco 2020; Enbody et al. 2022; Hale et al 2022; Khalil et al. 2023; Robinson et al. 2023) and therefore represent a promising framework for assessing the extent to which hormonal pleiotropy is conserved or labile across species.

In this study, we manipulated circulating testosterone levels of juvenile females and males from three species of *Sceloporus* lizards to simultaneously test for both sex- and species-specific effects of testosterone on the liver transcriptome. We used juveniles to test for effects on gene expression before pronounced sexual divergence in circulating testosterone levels during maturation, thereby avoiding potential confounding effects of sex differences in endogenous testosterone. We used liver because it

integrates growth and metabolism during development and exhibits a robust transcriptomic response to testosterone in lizards (Cox et al. 2017; Hale et al. 2022). Among the species we used in this study, *S. undulatus* and *S. virgatus* adults have female-biased sexual size dimorphism, while *S. merriami* has male-biased sexual size dimorphism, which could be associated with evolutionary changes in effects of testosterone on the expression of growth regulatory genes in the liver (Duncan et al. 2020). However, our study is not intended to link transcriptome to organismal phenotype, or to directly test the organization activation hypothesis or the evolutionary potential constraint hypothesis per se, but to provide a framework for assessing the sex and species specificity of hormonally mediated gene expression in a way that advances our understanding of each. If the sexes differ in early organizational effects of hormones or in other regulatory features that mediate responsiveness to elevated testosterone later in life, then we predict that (1) females and males will differ in the number and identity of differentially expressed genes, (2) transcriptome-wide correlations in the responsiveness of individual genes to testosterone will be low between the sexes, and (3) differentially expressed genes will exhibit sex-by-treatment interactions. If species-specific patterns of hormonal regulation have evolved, then we predict that (1) species will differ in the number and identity of differentially expressed genes, (2) transcriptome-wide correlations in the responsiveness of individual genes to testosterone will be low between species, (3) differentially expressed genes will exhibit species-by-treatment interactions, and (4) these patterns will be most pronounced between phylogenetically distant species with different patterns of sexual dimorphism.

Methods

Experimental Design and Sample Collection

We characterized responsiveness to testosterone in three *Sceloporus* species: closely related *S. undulatus* and *S. virgatus*, which diverged ~12 million years ago, and more distantly related *S. merriami*, which diverged from the other two species ~30 million years ago (Wiens 1999; Leaché et al. 2016; Ossip-Drahos et al. 2016). We collected wild juveniles at approximately 1 month of age in the late summer or early fall (for sampling locations and dates, see table S1; tables S1–S16 are available online), depending on the reproductive phenology of each species. Although the timing of sexual maturity varies across species, it is not achieved until the following spring at the earliest, with some individuals and species delaying maturity until their second spring (Dunham 1981; Ballinger and Ketels 1983; Haenel and John-Alder 2002). After 1 month of acclimation in captivity, females and males of each species were split into two treatment groups. One treatment group received an intraperitoneal Silastic implant containing 100 μg of crystalline testosterone that was designed to consistently elevate circulating testosterone levels for the duration of the experiment, and the other treatment group received an empty implant as a control. Implant construction and surgical procedures followed previous studies (Cox et al. 2015, 2017; Wittman et al. 2021; Robinson et al. 2023) and are described in the supplemental PDF. Because our experiment was designed to simultaneously assess androgen-mediated growth regulation and development of coloration as part of separate studies (e.g., Robinson et al. 2023), we allowed animals to grow for 8 weeks under experimental conditions (for details on animal husbandry, see the supplemental PDF). Hormone implants can lead to unintended physiological consequences as hormonally mediated feedback loops adapt to chronic elevation of the hormone (Fusani et al. 2007; Fusani 2008; Gerald et al. 2022). For example, elevated androgen levels can up- or downregulate androgen receptor expression in a tissue-dependent manner (Hunter et al. 2018), resulting in increased or decreased androgen sensitivity. While previous work in other lizard species suggests that our implant method induces many of the same patterns of gene expression that characterize natural age and sex differences in the liver transcriptome (Cox et al. 2017; Hale et al. 2022), it is still important to note that long-term transcriptomic effects of chronically elevated testosterone may differ from short-term effects induced by natural diel cycles or acute experimental manipulations.

After 8 weeks, we euthanized each animal via decapitation and immediately collected blood to confirm treatment effects on circulating testosterone levels via radioimmunoassay (see the supplemental PDF). We also immediately collected liver samples into RNAlater stabilization solution (ThermoFisher Scientific) on ice, refrigerated them for 24 h at 4°C, and stored them at −80°C until RNA extraction. We focused on gene expression in the liver because it responds to testosterone and androgen-mediated signals, such as growth hormone, and has been shown to diverge between the sexes in response to androgens across ontogeny (Cox et al. 2017; Hale et al. 2022).

RNA Extraction and Sequencing

We extracted RNA from livers of 72 juvenile lizards (median $n = 6$ per treatment per sex per species; for exact sample sizes in each group, see table 1) using illustra RNAspin mini RNA isolation kits (GE Healthcare) following manufacturer specifications, with detailed procedures and slight modifications described in the supplemental PDF. Library preparation and sequencing were carried out by the Georgia Genomics and Bioinformatics Core (University of Georgia, Athens, GA). RNA quality

Table 1: Sample sizes for analysis in each species, sex, and treatment group

	Control			Testosterone			
Species	Females	Males	Total	Females	Males	Total	Genes retained
Sceloporus undulatus	6	6	12	6	7	13	13,891
S. virgatus	6	6	12	6	6	12	13,772
S. merriami	6	5	11	5	5	10	13,036

Note: We extracted RNA from liver from 72 individuals, but two *S. merriami* libraries in the testosterone treatment group (one female, one male) were removed from analyses (not included here) because they were subsequently determined to have implants that were exhausted. The number of genes retained for analysis after filtering for low expression is indicated for each species.

was assessed using an Agilent 2100 BioAnalyzer. Complementary DNA libraries were prepared from total RNA (~500 ng per sample) using KAPA Biosystems (Wilmington, MA) RNA library preparation chemistry with poly(A) selection. Libraries were sequenced on an Illumina NextSeq 2000 (2 × 100 bp paired-end sequencing) using P3 high-output flow cells. We assessed read quality and trimmed reads using Fastp (Chen et al. 2018), then aligned reads to the *S. undulatus* genome (Westfall et al. 2021; GCA_019175285.1, SceUnd_v1.1) using subread-align (Liao et al. 2013), with *S. undulatus* transcripts as an alignment guide (GCF_019175285.1). Although the proportion of reads from other species that map to the *S. undulatus* genome declines with phylogenetic distance, this should not introduce any systematic bias to our estimation of sex or treatment differences in gene expression, since any mapping issues would be common to either sex or treatment group of a species. Following alignment, we assigned uniquely mapped fragments to annotated *S. undulatus* genes using featureCounts (Liao et al. 2014) to generate a matrix of read counts. We summed counts for each gene across paired and unpaired reads within each library. Many genes on the *S. undulatus* X chromosome (chromosome 10 in Westfall et al. 2021) have consistently higher expression in females than in males (M. D. Hale, C. D. Robinson, R. M. Cox, unpublished data). Therefore, we excluded all genes from chromosome 10 and unplaced scaffolds to focus on the effects of testosterone on autosomal genes that are present in equal doses in both sexes. Reads are available under accession number PRJNA1051777 at the National Center for Biotechnology Information Short Read Archive.

Analyses of Gene Expression

We excluded two *S. merriami* individuals in the testosterone treatment group (one female, one male) from our gene expression analyses because their plasma testosterone levels were no longer elevated at the time of tissue collection, suggesting that their implants had exhausted (see the supplemental PDF). To test for sex, treatment, and species effects on gene expression, we conducted differential gene expression analyses on read counts using the package edgeR (ver. 3.38.4; Robinson et al. 2010) in R (R Core Team 2022). Unless otherwise noted, we processed data independently for each species. To remove genes with low expression, we used filterByExpr in edgeR, retaining between 13,036 and 13,891 genes for each species (table 1). We then used robpca in the rospca package (ver. 1.0.4; Reynkens 2018) to conduct principal components analyses to test for outlier libraries, of which there were none. We normalized read counts using trimmed mean of *M* values normalization, then used glmQLFit in edgeR to fit a negative binomial model to our data, specifying robust = TRUE to reduce the influence of hypervariable genes (Phipson et al. 2016). We then used glmQLFTest to calculate quasi-likelihood *F*-tests for paired contrasts (e.g., control vs. testosterone treatment, female vs. male). We identified differentially expressed genes (DEGs) for each contrast as those with a Benjamini-Hochberg-corrected $P < .05$ (Benjamini and Hochberg 1995).

To characterize natural sex differences in gene expression, which are typically minor in juvenile lizards (Cox et al. 2017, 2022*a*; Hale et al. 2022; Robinson et al. 2023), we first identified genes that were differentially expressed between control females and control males of each species. We view these comparisons as descriptions of natural sex differences in gene expression, not as tests of our primary hypotheses. For comparison, we also identified genes that were differentially expressed between testosterone-treated females and testosterone-treated males of each species.

To test for sex differences in transcriptomic responses to testosterone in each species, we first identified genes that were differentially expressed between control and testosterone treatments within each sex. We then used χ^2 tests with 1 degree of freedom to test whether females and males of each species differed in the number of genes upregulated by testosterone, downregulated by testosterone, and either up- or downregulated by testosterone. Next, we combined both sexes into a single model for each species and used glmQLFTest in edgeR to identify genes with a main effect of treatment on expression and to test for genes in which the response to testosterone in one sex was different from the response to testosterone in the other, as indicated by a sex-by-treatment interaction. As a measure of the overall

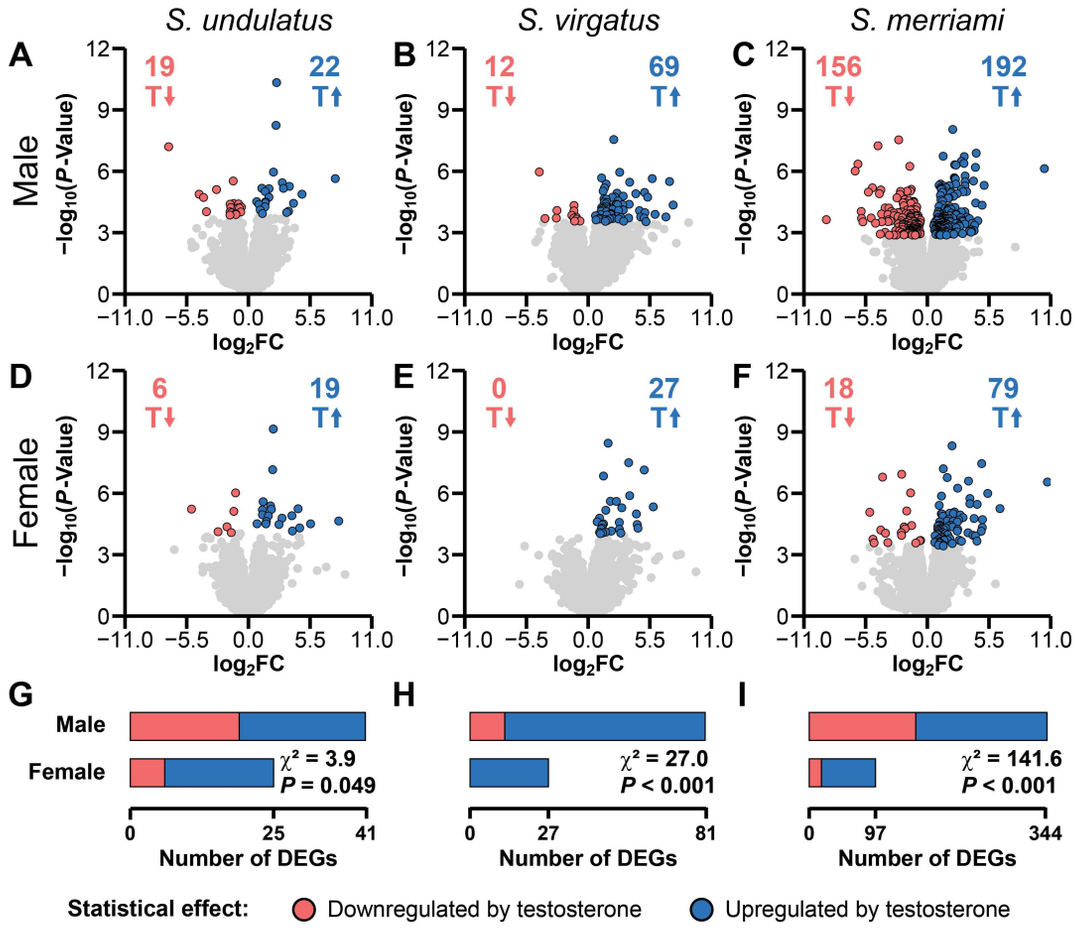


Figure 1: Volcano plots of the $-\log_{10} P$ value for the effect of testosterone on gene expression against the $\log_2$ fold change (FC) between testosterone and control groups for males (*A–C*) and females (*D–F*) in three species: *Sceloporus undulatus* (*left*), *S. virgatus* (*middle*), and *S. merriami* (*right*). Each plot represents output from sex- and species-specific models. Points represent individual genes, and positive values along the *x*-axis represent genes that are more highly expressed in the testosterone group (upregulated by testosterone), whereas negative values represent genes that are more highly expressed in the control group (downregulated by testosterone). Colored points represent genes that are significantly differentially expressed between treatment groups after *P* value correction, and the number of significantly up- and downregulated genes is presented in the upper corners of each plot. *G–I* show χ^2 tests for sex differences in the total number of differentially expressed genes alongside quantitative summaries of the number of up- and downregulated genes in each sex.

similarity of testosterone-mediated gene expression between the sexes, we regressed $\log_2$ fold change (the fold difference in mean expression between testosterone and control groups; hereafter, $\log_2$ FC) in females against the same measure of $\log_2$ FC in males, each estimated from sex-specific models. We interpreted the correlation coefficients from these regressions as measures of the overall similarity of transcriptomic responsiveness to testosterone between sexes.

To test whether the transcriptomic effects of testosterone are conserved across species, we used an omnibus differential gene expression model that simultaneously analyzed read counts from all three species. Therefore, we repeated gene filtering, normalization, fitting of a negative binomial model, and calculations of quasi-likelihood *F*-tests for all 70 libraries. This method retained 15,234 genes for analysis. For each species, we estimated the $\log_2$ FC between control and testosterone groups for each gene retained in the omnibus model, then regressed $\log_2$ FC values estimated from one species against those estimated from another species. We interpreted the correlation coefficients from these regressions as measures of the overall similarity of transcriptomic responsiveness to testosterone between species. Next, we tested for effects of testosterone on gene expression between species pairs by pooling data from two species and testing for differential gene expression between control

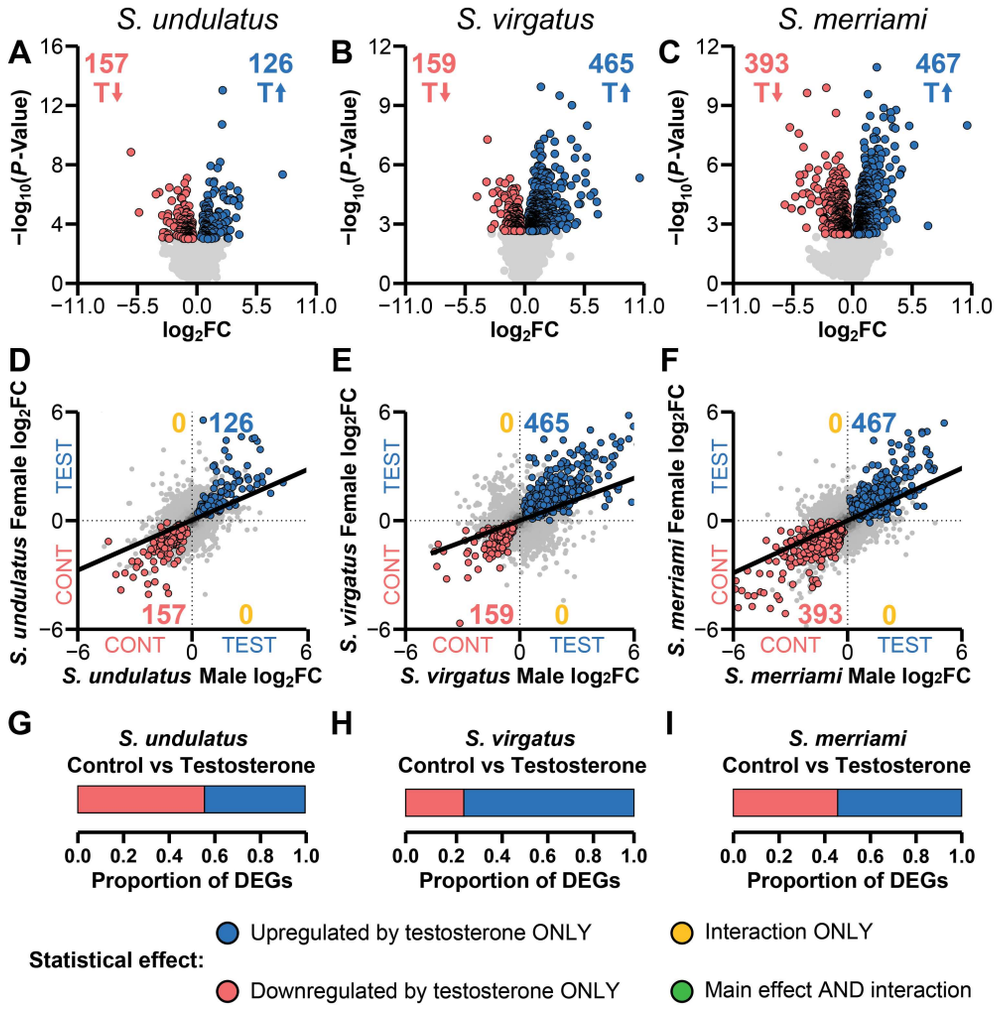


Figure 2: *A–C*, Volcano plots of the $-\log_{10} P$ value for the effect of testosterone on gene expression against the $\log_2$ fold change (FC) between testosterone and control groups for *Sceloporus undulatus* (*A*), *S. virgatus* (*B*), and *S. merriami* (*C*). Each plot represents output from species-specific models where sexes are combined. Points represent individual genes, and positive values along the *x*-axis represent genes that are more highly expressed in the testosterone group (upregulated by testosterone), whereas negative values represent genes that are more highly expressed in the control group (downregulated by testosterone). Colored points represent genes that are significantly differentially expressed between treatment groups after Benjamini-Hochberg correction, and the number of significantly up- and downregulated genes is represented in the upper corners of each plot. *D–F*, Relationship between male and female response to testosterone ($\log_2$ FC) for *S. undulatus* (*D*), *S. virgatus* (*E*), and *S. merriami* (*F*), where the $\log_2$ FC for each sex is estimated from sex-specific models. Blue and red points represent autosomal genes that have a significant effect of testosterone across sexes (same colored points from *A–C*). No genes have a significant sex-by-treatment interaction. *G–I*, Bars representing the proportion of genes that are significantly upregulated (blue) or downregulated (red) by testosterone out of the total number of differentially expressed genes (DEGs) for *S. undulatus* (*G*), *S. virgatus* (*H*), and *S. merriami* (*I*). TEST = testosterone implant; CONT = control implant.

and testosterone groups, constituting a main effect of treatment. We conducted a second analysis to test for differential gene expression with respect to the interaction between species and treatment. Combining these analyses allowed us to identify genes with an overall main effect of treatment (i.e., up- or downregulated by testosterone), genes with an interaction between species and treatment (i.e., genes differentially regulated by testosterone in each species), and genes with both a main effect and an interaction (i.e., genes in which the main effect of testosterone is driven by its responsiveness in only one species). We conducted each of the above analyses separately for each sex and again with sexes pooled. We used a three-proportions *Z*-test to examine whether the proportion of genes exhibiting a significant

Table 2: Correlation coefficients for the effects of testosterone on differential gene expression (log_2 fold change) estimated between sexes of a species or between species (estimated separately for females, males, and both sexes pooled)

Between sexes		Between species			
Species	*r*	Species pair	*r* in females	*r* in males	*r* in both
Sceloporus undulatus	.461**	*S. undulatus* vs. *S. virgatus*	.131**	.178**	.285**
S. virgatus	.450**	*S. undulatus* vs. *S. merriami*	.141**	.172**	.202**
S. merriami	.551**	*S. virgatus* vs. *S. merriami*	.015	.154**	.141**

** $P < .001$.

species-by-treatment interaction differed between species pairs, with pairwise, two-proportion *Z*-tests with Holm correction (Holm 1979) as post hoc tests. To explore the functions of testosterone-responsive genes, we used gene ontology analysis (see the supplemental PDF).

Results

Treatment Effects on Circulating Testosterone

Implants elevated plasma testosterone concentrations (treatment: $F_{1, 55} = 192.2$, $P < .001$; fig. S1, available online), with no effect of species ($F_{2, 55} = 0.546$, $P = .582$) or sex ($F_{1, 55} = 0.895$, $P = .348$) and no two- or three-way interactions (supplemental PDF). This suggests that any observed differences in testosterone-mediated gene expression were largely driven by how the sexes and species responded to testosterone and not by differences in their induced testosterone levels.

Sex Differences in Gene Expression

Sex differences in juvenile gene expression were almost entirely absent when comparing control males and females in *Sceloporus undulatus* (one DEG), *S. virgatus* (zero DEGs), and *S. merriami* (zero DEGs). Likewise, we did not detect any sex-biased genes when comparing juvenile females and males that received testosterone implants in each species.

Sex Differences in Effects of Testosterone on Gene Expression

In all three species, significantly more genes were differentially expressed in response to testosterone in males than in females (fig. 1; table S2). However, the overall direction of transcriptomic response to testosterone was highly concordant between sexes in each species (fig. 2), with significant correlations in log_2 FC between females and males (all $r > 0.45$, all $P < .001$; table 2). While the total numbers (fig. 2*A*–2*F*), relative proportions (fig. 2*G*–*I*), and individual identities of up- and downregulated genes differed across species, no genes exhibited a significant sex-by-treatment interaction in any species, indicating that female and male conspecifics responded similarly to testosterone (fig. 2*D*–2*F*). Within a species, 48%–62% of genes that were responsive to testosterone in females were also responsive to testosterone in males (table S3).

Species Differences in Effects of Testosterone on Gene Expression

In each species pair, testosterone consistently up- or downregulated hundreds of genes in the same direction for both species (67%–93% of all DEGs; fig. 3). However, in contrast to between-sex comparisons, between-species comparisons also revealed many genes that responded differently to testosterone (i.e., species-by-treatment interactions, 7%–33% of all DEGs; fig. 3). Some of these genes retained a main effect of treatment, indicating a species difference primarily in the magnitude of the response to testosterone (3%–13% of all DEGs; fig. 3), while others exhibited an interaction with no main effect, indicating a species difference in the direction of the response to testosterone (3%–21% of all DEGs; fig. 3). The number and proportion of DEGs exhibiting species-by-treatment interactions were relatively low between closely related *S. undulatus* and *S. virgatus* but high in either pairwise comparison involving more distantly related *S. merriami* (three-proportions *Z*-test: $\chi^2 = 307.66$, $P < .001$; fig. 3; table 3). Likewise, transcriptome-wide correlations for responsiveness to testosterone were much lower for between-species comparisons (all $r < 0.29$) than for between-sex comparisons (all $r > 0.45$; table 2). These correlations were higher between closely related *S. undulatus* and *S. virgatus* than between either of these two species and more distantly related *S. merriami* (fig. 3; table 2). Across species pairs, only 14%–26% of genes responsive to testosterone in one species were similarly responsive to testosterone in the other (table S8). Species also differed in the number of genes that were differentially expressed in response to testosterone, both within sexes (fig. 1) and when pooling sexes (fig. 2), although the biological reasons why *S. merriami* exhibited a stronger response than *S. virgatus* and *S. undulatus* are unclear.

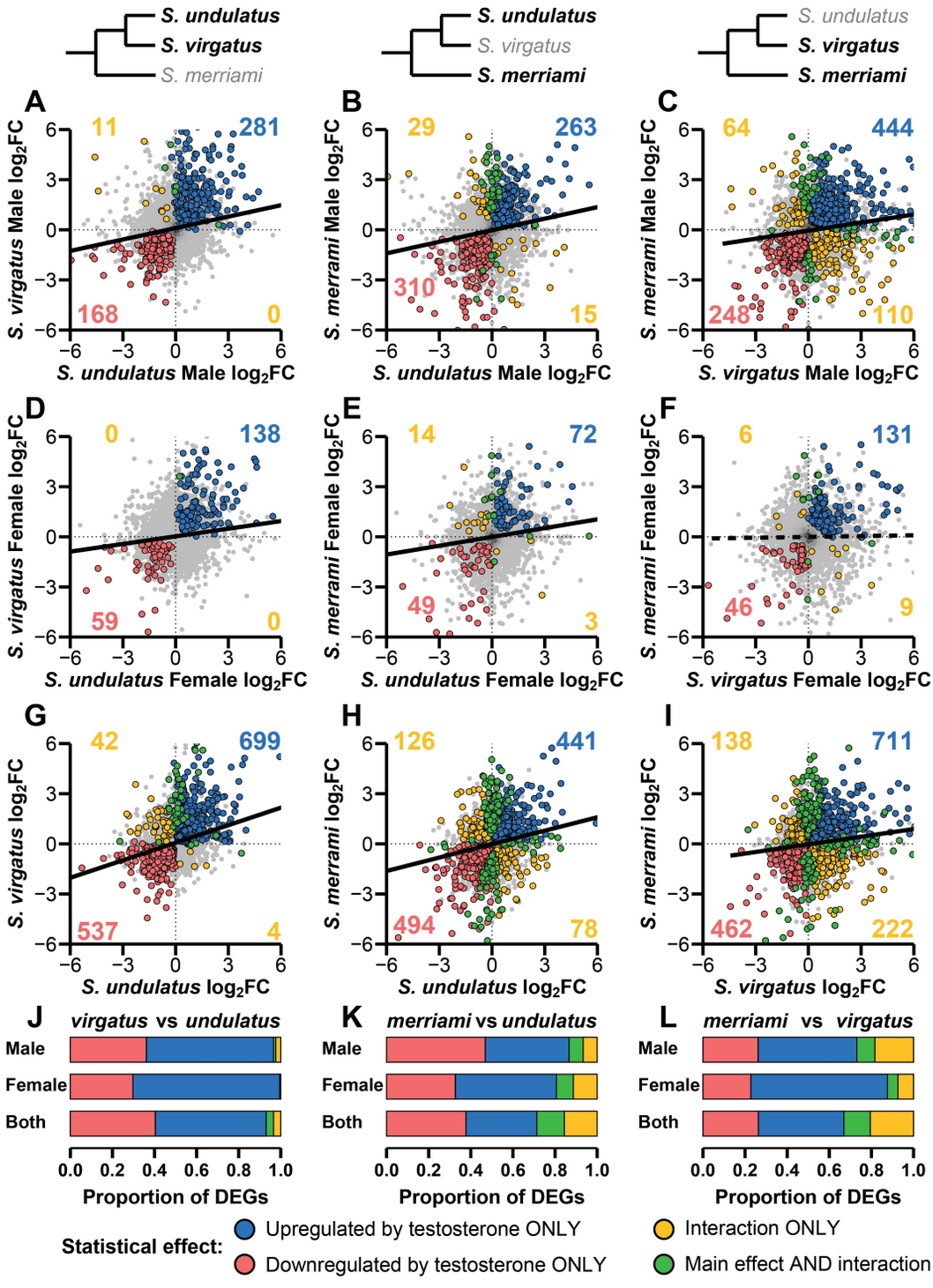


Figure 3: Relationship between experimentally induced gene expression across pairwise combinations of species, assessed for males (*A–C*), females (*D–F*), and both sexes (*G–I*). Phylogenies above each column represent the relationships among species, with species names in bold indicating the two species included in the pairwise comparison represented in that column. For all axes in *A–I*, $\log_2$ fold change (FC) represents the $\log_2$ FC of gene expression of individuals receiving a testosterone implant relative to individuals receiving a control implant from an omnibus model containing all three species. Positive values indicate that a gene is upregulated by testosterone, while negative values indicate that a gene is downregulated by testosterone. Genes with a significant main effect of treatment or a species-by-treatment interaction after *P* value correction are represented by colored points. The line represents the slope from a linear regression, showing the relationship of

Table 3: Results from post hoc analyses after a significant three-proportions *Z*-test examining whether the proportion of genes exhibiting a significant species-by-treatment interaction differed between species pairs

Species pair 1	Pair 1 DEGs	Species pair 2	Pair 2 DEGs	χ^2	P_{adj}
Sceloporus undulatus vs. *S. virgatus*	92 (.067)	*S. undulatus* vs. *S. merriami*	375 (.286)	211.63	<.001
S. undulatus vs. *S. virgatus*	92 (.067)	*S. virgatus* vs. *S. merriami*	579 (.330)	300.94	<.001
S. undulatus vs. *S. merriami*	375 (.286)	*S. virgatus* vs. *S. merriami*	579 (.330)	6.63	.010

Note: The number of differentially expressed genes (DEGs) with a significant interaction is represented in the "Pair 1 DEGs" and "Pair 2 DEGs" columns. Values in parentheses represent the proportion of genes with an interaction out of the total number of genes with a significant main effect or interaction. χ^2 values are from post hoc two-proportion *Z*-tests, and P_{adj} represents the adjusted *P* value after Holm correction.

Discussion

Exogenous testosterone induced significantly more DEGs in juvenile males than in juvenile females in each of three *Sceloporus* lizard species. Yet the overall effects of testosterone on the liver transcriptome were highly concordant between sexes. Furthermore, no gene exhibited a significant sex-by-treatment interaction in any species, suggesting that testosterone regulates autosomal gene expression similarly in juvenile females and males, albeit to a greater degree in males. In contrast, many genes exhibited significant species differences in their response to testosterone, particularly between distantly related species, indicating that the regulatory coupling of testosterone to gene expression has evolved across species. These results suggest that early organizational effects may predispose males to (or prevent females from) enhanced transcriptomic responsiveness to testosterone later in life and that the evolutionary lability of hormonally regulated gene expression may facilitate phenotypic diversification in closely related species. We note that, because treatment effects on circulating testosterone levels were equivalent between sexes and across species, any observed sex or species differences in treatment effects on the transcriptome likely reflect differences in tissue sensitivity and gene regulatory mechanisms, rather than differences in circulating testosterone levels.

In each *Sceloporus* species, we found that significantly more genes were both up- and downregulated by testosterone in males than in females. A similar sex difference in the number of DEGs was observed in the liver transcriptome of another lizard, *Anolis sagrei*, following treatment of juveniles with exogenous testosterone (Hale et al. 2022). In *A. sagrei*, exogenous testosterone masculinizes juvenile female phenotypes (Cox et al. 2015), statistical patterns of phenotypic and genetic covariance (Cox 2020; Wittman et al. 2021), and underlying gene expression (Cox et al. 2017; Hale et al. 2022). Likewise, in *Sceloporus*, treatment of juvenile females with testosterone masculinizes ventral coloration (Cox et al. 2005*b*) and induces the transcription of underlying genes for melanin synthesis in the ventral skin (Robinson et al. 2023). Collectively, these studies indicate that phenotypic and transcriptomic effects of testosterone are broadly similar in juveniles of either sex but that a larger portion of the transcriptome is responsive to testosterone in males (i.e., more genes are differentially expressed). Moreover, the between-sex correlation in overall transcriptomic responsiveness to testosterone was high in each *Sceloporus* species (table 2), and no genes exhibited sex-by-treatment interactions (fig. 3). This stands in contrast to results from testosterone manipulation in dark-eyed juncos (*Junco hyemalis*), where hundreds of genes exhibited sex-by-treatment interactions in brain, liver, and muscle (Peterson et al. 2013, 2014). Whereas we treated juvenile females and males with identical doses of testosterone that approximated levels in adult males, adult female and male juncos were treated with different doses that approximated the respective adult maxima for each sex. Therefore, Peterson et al. (2013, 2014) observed sex-specific transcriptomic responses to testosterone when using sex-specific doses in sexually dimorphic adults, whereas we observed broadly concordant transcriptomic responses when using identical doses before the development of pronounced sexual dimorphism in juveniles.

Several mechanisms could explain why transcriptomic responses to testosterone are greater in juvenile males than in juvenile females in *Sceloporus*. For example, sexes could differ in androgen receptor density, transcriptional cofactor availability, binding globulins, or chromatin accessibility in the liver and other tissues (Cox 2020). Such sex differences in hormonal sensitivity could arise through early organizational effects of hormones that shape transcriptomic responses to testosterone later in life (Phoenix 1959; Dufty et al. 2002; Adkins-Regan 2007; Anderson et al. 2022). Typically, investigations into the organizing effects of sex hormones focus on behavior (Phoenix et al. 1959; McCarthy

testosterone-induced gene expression across the entire liver transcriptome in pairwise comparisons. *A–C* represent models that include only males, *D–F* represent models that include only females, and *G–I* represent models that include both sexes. Values in *A–I* represent the number of genes with that effect. Genes with a significant main effect and interaction are not numerically represented in *A–I*. All regression lines are significant at $P < .001$ except in F ($P = .059$, dashed line). *J–L* summarize the proportion of differentially expressed genes (DEGs) for each statistical effect from all nine comparisons.

et al. 2009; McCarthy 2016). Although not linked to any specific organismal phenotype, our transcriptomic data suggest that similar organization may predispose males to stronger activational effects of testosterone, relative to females. Although our data do not clarify the underlying mechanisms that mediate this sex-specific sensitivity to testosterone or conclusively demonstrate that it arises from organizational effects of hormones per se, our results suggest that transcriptomes provide a promising means of directly testing for organizational and activational effects of hormones in future work.

Phenotypic diversification often involves the alteration of interactions between developmental regulators and genes (Carroll 1995, 2008; Chen and Rajewsky 2007; Prud'homme et al. 2007; Streisfeld and Rausher 2009; Romero et al. 2012; Sackton et al. 2019). Such regulatory changes can break phenotypic and genetic correlations, facilitating trait evolution (McGlothlin and Ketterson 2008; Rabinowitz and Vokes 2012; Tsuboi et al. 2018; Cox 2020; McGlothlin et al. 2022). The evolutionary constraint hypothesis proposes that tight coordination between hormones and the phenotypes they regulate limits diversification, while the evolutionary potential hypothesis proposes that downstream regulatory nodes of endocrine networks can evolve independently to limit the disruption of downstream phenotypes with shared regulatory components (Hau 2007). Although the dominant trend in our study was for genes to respond similarly to testosterone across *Sceloporus* species, we also observed many genes with species-specific responses to testosterone, suggesting that the relationship between testosterone and gene expression is evolutionary labile. Pairwise comparisons with distantly related *S. merriami* exhibited the highest proportion of genes with significant treatment-by-species interactions, as expected if changes in hormonal regulation accrue with evolutionary divergence. Because our treatments resulted in similar effects on circulating testosterone in each species (fig. S1), we can infer that species differences in transcriptomic responses to testosterone are not due to differences in circulating hormones but instead likely reflect the evolution of hormone-genome interactions (Cox et al. 2022*b*). In *Onthophagus* dung beetles, horn development involves *doublesex* (Kijimoto et al. 2012), Hedgehog signaling (Kijimoto et al. 2016), insulin signaling (Snell-Rood and Moczek 2012; Casasa and Moczek 2018), and serotonin signaling (Schwab et al. 2020). Interactions among these elements have evolved to result in novel transcriptomic regulation (Kijimoto et al. 2014; Ledón-Rettig and Moczek 2016; Ledón-Rettig et al. 2017) and different patterns of sexually dimorphic horn development (reviewed in Casasa et al. 2017). This illustrates how the principles of the evolutionary potential hypothesis extend beyond vertebrate-specific hormones such as testosterone to include other familiar examples in which relationships among the various nodes in a pleiotropically regulated endocrine network can evolve.

In *Sceloporus*, evolutionary changes in sexual dimorphism for phenotypes such as body size and coloration are associated with species differences in how underlying physiological processes such as growth and melanin synthesis respond to testosterone (Quinn and Hews 2003; Cox and John-Alder 2005; Cox et al. 2005*b*; John-Alder and Cox 2007). In the case of coloration, these species differences in sexual dimorphism have been directly linked to underlying species differences in the expression of melanin-synthesis genes in response to testosterone (Robinson et al. 2023). Although we do not link patterns of testosterone-mediated gene expression in the liver to organismal phenotypes, our results suggest that underlying changes in the response to testosterone can evolve for many individual genes, which may facilitate the evolution of phenotypic sexual dimorphism. For example, testosterone promotes growth and stimulates the expression of insulin-like growth factor genes *IGF1* and *IGF2* in the liver of an *Anolis* lizard species that exhibits pronounced male-biased sexual size dimorphism (Cox et al. 2017), whereas it inhibits growth and reduces the expression of *IGF1* in *Sceloporus* species that exhibit female-biased sexual size dimorphism (Cox and John-Alder 2005; Cox et al. 2005*a*; Duncan et al. 2020). Direct comparisons of monomorphic and dimorphic *Anolis* species also reveal sex-by-species interactions for *IGF1* and *IGF2* expression in the liver (Cox et al. 2022*a*), analogous to the treatment-by-species interactions we observed for many genes in the *Sceloporus* liver. Although many biosynthetic and metabolic processes were enriched for genes with opposite responses to testosterone in *S. merriami* (males larger) relative to *S. undulatus* and *S. virgatus* (females larger; see "Supplementary Methods and Results" in the supplemental PDF; tables S15, S16), further work is required to explore whether these divergent transcriptomic responses to testosterone are associated with the evolution of sexually dimorphic phenotypes such as growth and body size.

The development of sexual dimorphism requires regulatory mechanisms that permit sex-specific expression of a shared autosomal genome, and the evolution of sexual dimorphism requires that these regulatory mechanisms can be modified in species-specific ways. While much previous work has focused on how sexual dimorphism arises from sex differences in circulating hormone levels, ours is one of the few studies to test whether the sexes also respond differently to the same hormonal signal (Peterson et al. 2013, 2014; Mittal et al. 2021). Our results suggest that early developmental processes prime the sexes for differences in the magnitude, but not the direction, of transcriptomic response to testosterone, as expected if early organizational effects set the boundaries for responses to later activational effects of sex steroids (Dufty et al. 2002). Likewise, previous

studies indicate that evolutionary changes in the magnitude of sexual dimorphism can be achieved by species-specific changes to circulating hormone levels (Husak and Lovern 2014; Swanson and Dantzer 2014; Karagic et al. 2022), but ours is one of the few studies to directly test whether species differ in their transcriptomic response to the same hormonal signal (Robinson et al. 2023). We find that species differences in the response to testosterone increase with phylogenetic distance and when comparing species with different patterns of sexual size dimorphism. Although we cannot distinguish the relative contributions of these two factors, either would be consistent with the interpretation that many of the regulatory couplings between hormones and genes evolve, thus indicating considerable evolutionary lability in the transcriptomic architecture of hormonal pleiotropy.

Acknowledgments

We thank the Georgia Genomics and Bioinformatics Core for library preparation and sequencing. We also thank Christian Jeitner for assistance with radioimmunoassays, Matt Watson and Tyler Wittman for field assistance, and Ashlyn Crain, Myles Davoll, Pietro de Mello, Daniel Nondorf, and Tyler Wittman for early reviews of this work. All procedures involving animals were approved by the University of Virginia's Animal Care and Use Committee (protocol 3896). Animals were collected under permits from New Jersey Fish and Wildlife (SC 2019007), Arizona Game and Fish (SP658032), the US Forest Service (Coronado National Forest), and Texas Parks and Wildlife (SPR-0719-113). This work was supported by a research traineeship award from the National Science Foundation (NSF NRT-ROL 2021791, training fellowship to C.D.R.), a collaborative research award from the National Science Foundation (IOS 1755134 and 2024064 to C.L.C., IOS 1754934 to H.B.J.-A., and IOS 1755026 to R.M.C.), and a McIntire-Stennis award (NJ17321 to H.B.J.-A).

Statement of Authorship

C.D.R. was responsible for conceptualization, data curation, formal analysis, investigation, visualization, writing the original draft, and reviewing and editing the manuscript. M.D.H. was responsible for methodology and reviewing and editing the manuscript. C.L.C. was responsible for funding acquisition, resources, and reviewing and editing the manuscript. H.B.J.-A. was responsible for funding acquisition, resources, and reviewing and editing the manuscript. R.M.C. was responsible for conceptualization, funding acquisition, resources, supervision, visualization, and reviewing and editing the manuscript. All authors gave final approval for publication and agreed to be held accountable for the work performed herein.

Data and Code Availability

Reads from RNAseq are available at the National Center for Biotechnology Information Short Read Archive (BioProject ID PRJNA1051777). Read counts and phenotypic data are available on the Dryad Digital Repository (https://doi.org/10.5061/dryad.fj6q57432; Robinson et al. 2024).

Literature Cited

Adkins-Regan, E. 2007. Hormones and the development of sex differences in behavior. Journal of Ornithology 148:17–26. https://doi.org/10.1007/s10336-007-0188-3.

———. 2012. Hormonal organization and activation: evolutionary implications and questions. General and Comparative Endocrinology 176:279–285. https://doi.org/10.1016/j.ygcen.2011.12.040.

Anderson, A. P., and S. C. P. Renn. 2023. The ancestral modulation hypothesis: predicting mechanistic control of sexually heteromorphic traits using evolutionary history. American Naturalist 202:241–259. https://doi.org/10.1086/725438.

Anderson, A. P., E. Rose, S. P. Flanagan, and A. G. Jones. 2020. The estrogen-responsive transcriptome of female secondary sexual traits in the gulf pipefish. Journal of Heredity 111:294–306. https://doi.org/10.1093/jhered/esaa008.

Anderson, N. K., S. E. Goodwin, E. R. Schuppe, A. Dawn, D. Preininger, L. A. Mangiamele, and M. J. Fuxjager. 2022. Activational vs. organizational effects of sex steroids and their role in the evolution of reproductive behavior: looking to foot-flagging frogs and beyond. Hormones and Behavior 146:105248. https://doi.org/10.1016/j.yhbeh.2022.105248.

Anderson, N. K., E. R. Schuppe, K. V. Gururaja, L. A. Mangiamele, J. C. C. Martinez, H. Priti, R. von May, D. Preininger, and M. J. Fuxjager. 2021. A common endocrine signal marks the convergent evolution of an elaborate dance display in frogs. American Naturalist 198:522–539. https://doi.org/10.1086/716213.

Arnold, A. P. 2009. The organizational-activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. Hormones and Behavior 55:570–578. https://doi.org/10.1016/j.yhbeh.2009.03.011.

Arnold, A. P., and S. M. Breedlove. 1985. Organizational and activational effects of sex steroids on brain and behavior: a reanalysis. Hormones and Behavior 19:469–498. https://doi.org/10.1016/0018-506X(85)90042-X.

Ballinger, R. E., and D. J. Ketels. 1983. Male reproductive cycle of the lizard *Sceloporus virgatus*. Journal of Herpetology 17:99–102. https://doi.org/10.2307/1563795.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society B 57:289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

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

Bodo, C., and E. F. Rissman. 2008. The androgen receptor is selectively involved in organization of sexually dimorphic social behaviors in mice. Endocrinology 149:4142–4150. https://doi.org/10.1210/en.2008-0183.

Carroll, S. B. 1995. Homeotic genes and the evolution of arthropods and chordates. Nature 376:479–485. https://doi.org/10.1038/376479a0.

———. 2008. Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. Cell 134:25–36. https://doi.org/10.1016/j.cell.2008.06.030.

Casasa, S., and A. P. Moczek. 2018. Insulin signalling's role in mediating tissue-specific nutritional plasticity and robustness in the horn-polyphenic beetle *Onthophgus taurus*. Proceedings of the Royal Society B 285:20181631. https://doi.org/10.1098/rspb.2018.1631.

Casasa, S., D. B. Schwab, and A. P. Moczek. 2017. Developmental regulation and evolution of scaling: novel insights through the study of *Onthophagus* beetles. Current Opinion in Insect Science 19:52–60.

Chen, K., and N. Rajewsky. 2007. The evolution of gene regulation by transcription factors and microRNAs. Nature Reviews Genetics 8:93–103. https://doi.org/10.1038/nrg1990.

Chen, S., Y. Zhou, Y. Chen, and J. Gu. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890. https://doi.org/10.1093/bioinformatics/bty560.

Chinnathambi, V., C. Yallampalli, and K. Sathishkumar. 2013. Prenatal testosterone induces sex-specific dysfunction in endothelium-dependent relaxation pathways in adult male and female rates. Biology of Reproduction 89:1–9. https://doi.org/10.1095/biolreprod.113.111542.

Cox, C. L., A. F. Hanninen, A. M. Reedy, and R. M. Cox. 2015. Female anoles retain responsiveness to testosterone despite the evolution of androgen-mediated sexual dimorphism. Functional Ecology 29:758–767. https://doi.org/10.1111/1365-2435.12383.

Cox, C. L., M. L. Logan, D. J. Nicholson, A. K. Chung, A. A. Rosso, W. O. McMillan, and R. M. Cox. 2022*a*. Species-specific expression of growth-regulatory genes in 2 anoles with divergent patterns of sexual size dimorphism. Integrative Organismal Biology 4:obac025. https://doi.org/10.1093/iob/obac025.

Cox, R. M. 2020. Sex steroids as mediators of phenotypic integration, genetic correlations, and evolutionary transitions. Molecular and Cellular Endocrinology 502:110668. https://doi.org/10.1016/j.mce.2019.110668.

Cox, R. M., C. L. Cox, J. W. McGlothlin, D. C. Card, A. L. Andrew, and T. A. Castoe. 2017. Hormonally mediated increases in sex-biased gene expression accompany the breakdown of between-sex genetic correlations in a sexually dimorphic lizard. American Naturalist 189:315–332. https://doi.org/10.1086/690105.

Cox, R. M., M. D. Hale, T. N. Wittman, C. D. Robinson, and C. L. Cox. 2022*b*. Evolution of hormone-phenotype couplings and hormone-genome interactions. Hormones and Behavior 114:105216. https://doi.org/10.1016/j.yhbeh.2022.105216.

Cox, R. M., and H. B. John-Alder. 2005. Testosterone has opposite effects on male growth in lizards (*Sceloporus* spp.) with opposite patterns of sexual size dimorphism. Journal of Experimental Biology 208:4679–4687. https://doi.org/10.1242/jeb.01948.

Cox, R. M., S. L. Skelly, and H. B. John-Alder. 2005*a*. Testosterone inhibits growth in juvenile male eastern fence lizards (*Sceloporus undulatus*): implications for energy allocation and sexual size dimorphism. Physiological and Biochemical Zoology 78:531–545. https://doi.org/10.1086/430226.

Cox, R. M., S. L. Skelly, A. Leo, and H. B. John-Alder. 2005*b*. Testosterone regulates sexually dimorphic coloration in the eastern fence lizard, *Sceloporus undulatus*. Copeia 2005:597–608. https://doi.org/10.1643/CP-04-313R.

Cox, R. M., D. S. Stenquist, and R. Calsbeek. 2009. Testosterone, growth and the evolution of sexual size dimorphism. Journal of Evolutionary Biology 22:1586–1598. https://doi.org/10.1111/j.1420-9101.2009.01772.x.

Cox, R. M., V. Zilberman, and H. B. John-Alder. 2008. Testosterone stimulates the expression of a social color signal in Yarrow's spiny lizard, *Sceloporus jarrovii*. Journal of Experimental Zoology A 309:505–514. https://doi.org/10.1002/jez.481.

Dantzer, B., and E. M. Swanson. 2017. Does hormonal pleiotropy shape the evolution of performance and life history traits? Integrative and Comparative Biology 57:372–384. https://doi.org/10.1093/icb/icx064.

Davidson, P. L., E. M. Nadolski, and A. P. Moczek. 2023. Gene regulatory networks underlying the development and evolution of plasticity in horned beetles. Current Opinion in Insect Science 60:101114. https://doi.org/10.1016/j.cois.2023.101114.

Dufty, A. M., J. Clobert, and A. P. Møller. 2002. Hormones, developmental plasticity and adaptation. Trends in Ecology and Evolution 17:190–196. https://doi.org/10.1016/S0169-5347(02)02498-9.

Duncan, C. A., W. S. Cohick, and H. B. John-Alder. 2020. Testosterone reduces growth and hepatic *IGF-1* mRNA in a female-larger lizard, *Sceloporus undulatus*: evidence of an evolutionary reversal in growth regulation. Integrative Organismal Biology 2:obaa036. https://doi.org/10.1093/iob/obaa036.

Dunham, A. E. 1981. Populations in a fluctuating environment: the comparative population ecology of the iguanid lizards *Sceloporus merriami* and *Urosaurus ornatus*. Miscellaneous Publications, Museum of Zoology, University of Michigan 158:1–72.

Enbody, E. D., S. Y. W. Sin, J. Boersma, S. V. Edwards, S. Ketaloya, H. Schwabl, M. S. Webster, and J. Karubian. 2022. The evolutionary history and mechanistic basis of female ornamentation in a tropical songbird. Evolution 76:1720–1736. https://doi.org/10.1111/evo.14545.

Fiber, J. M., and J. M. Swann. 1996. Testosterone differentially influences sex-specific pheromone-stimulated Fos expression in limbic regions of Syrian hamsters. Hormones and Behavior 30:455–473. https://doi.org/10.1006/hbeh.1996.0050.

Finseth, F. R., and R. G. Harrison. 2018. Genes integral to the reproductive function of male reproductive tissues drive heterogeneity in evolutionary rates in Japanese quail. G3: Genes, Genomes, Genetics 8:39–51. https://doi.org/10.1534/g3.117.300095.

Flatt, T., M.-P. Tu, and M. Tatar. 2005. Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history. BioEssays 27:999–1010. https://doi.org/10.1002/bies.20290.

Frankl-Vilches, C., H. Kuhl, M. Werber, S. Klages, M. Kerick, A. Bakker, E. H. C. de Oliveira, et al. 2015. Using the canary genome to decipher the evolution of hormone-sensitive gene regulation in seasonal singing birds. Genome Biology 16:19. https://doi.org/10.1186/s13059-014-0578-9.

Fusani, L. 2008. Endocrinology in field studies: problems and solutions for the experimental design. General and Comparative Endocrinology 157:249–253. https://doi.org/10.1016/j.ygcen.2008.04.016.

Fusani, L., L. B. Day, V. Canoine, D. Reinemann, E. Hernandez, and B. Schlinger. 2007. Androgen and the elaborate courtship behavior of a tropical lekking bird. Hormones and Behavior 51:62–68. https://doi.org/10.1016/j.yhbeh.2006.08.005.

Fuxjager, M. J., J. Eaton, W. R. Lindsay, L. H. Salwiczek, M. A. Rensel, J. Barske, L. Sorenson, L. B. Day, and B. A. Schlinger. 2015. Evolutionary patterns of adaptive acrobatics and physical performance predict expression profiles of androgen receptor—but not oestrogen receptor—in the forelimb musculature. Functional Ecology 29:1197–1208. https://doi.org/10.1111/1365-2435.12438.

Fuxjager, M. J., J.-H. Lee, T.-M. Chan, J. H. Bahn, J. G. Chew, X. Xiao, and B. A. Schlinger. 2016. Research resource: hormones, genes, and athleticism: effect of androgens on the avian muscular transcriptome. Molecular Endocrinology 30:254–271. https://doi.org/10.1210/me.2015-1270.

Fuxjager, M. J., M. C. Miles, and B. A. Schlinger. 2018. Evolution of the androgen-induced male phenotype. Journal of Comparative Physiology A 204:81–92. https://doi.org/10.1007/s00359-017-1215-3.

Fuxjager, M. J., and E. R. Schuppe. 2018. Androgenic signalling systems and their role in behavioral evolution. Journal of Steroid Biochemistry and Molecular Biology 184:47–56. https://doi.org/10.1016/j.jsbmb.2018.06.004.

Gegenhuber, B., and J. Tollkuhn. 2020. Signatures of sex: sex differences in gene expression in the vertebrate brain. Developmental Biology 9:e348. https://doi.org/10.1002/wdev.348.

Gerald, T., and G. Raj. 2022. Testosterone and the androgen receptor. Urologic Clinics of North America 49:603–614. https://doi.org/10.1016/j.ucl.2022.07.004.

Haenel, G. J., and H. B. John-Alder. 2002. Experimental and demographic analyses of growth rate and sexual size dimorphism in a lizard, *Sceloporus undulatus*. Oikos 96:70–81. https://doi.org/10.1034/j.1600-0706.2002.10915.x.

Hale, M. D., C. D. Robinson, C. L. Cox, and R. M. Cox. 2022. Ontogenetic change in male expression of testosterone-responsive genes contributes to the emergence of sex-biased gene expression in *Anolis sagrei*. Frontiers in Physiology 13:886973. https://doi.org/10.3389/fphys.2022.886973.

Hau, M. 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. BioEssays 29:133–144. https://doi.org/10.1002/bies.20524.

Hau, M., and J. C. Wingfield. 2011. Hormonally-regulated trade-offs: evolutionary variability and phenotypic plasticity in testosterone signalling pathways. Pages 349–361 *in* T. Flatt and A. Heyland, eds. Mechanisms of life history evolution. Oxford University Press, Oxford. https://hdl.handle.net/11858/00-001M-0000-0028-48B8-1.

Hews, D. K., and M. C. Moore. 1995. Influence of androgens on differentiation of secondary sex characters in tree lizards, *Urosaurus ornatus*. General and Comparative Endocrinology 97:86–102. https://doi.org/10.1006/gcen.1995.1009.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6:65–70. https://www.jstor.org/stable/4615733.

Hunter, I., C. W. Hay, B. Esswein, K. Watt, and I. J. McEwan. 2018. Tissue control of androgen action: the ups and downs of androgen receptor expression. Molecular and Cellular Endocrinology 465:27–35. https://doi.org/10.1016/j.mce.2017.08.002.

Husak, J. F., and M. B. Lovern. 2014. Variation in steroid hormone levels among Caribbean *Anolis* lizards: endocrine system convergence? Hormones and Behavior 65:408–415. https://doi.org/10.1016/j.yhbeh.2014.03.006.

John-Alder, H. B., and R. M. Cox. 2007. Development of sexual size dimorphism in lizards: testosterone as a bipotential growth regulator. Pages 195–204 *in* D. J. Fairbairn, W. U. Blanckenhorn, and T. Szekely, eds. Sex, size and gender roles: evolutionary studies of sexual size dimorphism. Oxford University Press, London.

Johnson, M. A., B. K. Kircher, and D. J. Castro. 2018. The evolution of androgen receptor expression and behavior in *Anolis* lizard forelimb muscles. Journal of Comparative Physiology A 204:71–79. https://doi.org/10.1007/s00359-017-1228-y.

Karagic, N., A. Härer, A. Meyer, and J. Torres-Dowdall. 2022. Thyroid hormone tinkering elicits integrated phenotypic changes potentially explaining rapid adaptation of colour vision in cichlid fish. Evolution 76:837–845. https://doi.org/10.1111/evo.14455.

Ketterson, E. D., J. W. Atwell, and J. W. McGlothlin. 2009. Phenotypic integration and independence: hormones, performance, and response to environmental change. Integrative and Comparative Biology 49:365–379. https://doi.org/10.1093/icb/icp057.

Ketterson, E. D, and V. Nolan Jr. 1999. Adaptation, exaptation, and constraint: a hormonal perspective. American Naturalist 154 (suppl.):S4–S25. http://www.jstor.org/stable/10.1086/303280.

Khalil, S., E. D. Enbody, C. Frankl-Vilches, J. F. Welklin, R.E. Koch, M. B. Toomey, S. Y. W. Sin, et al. 2023. Testosterone coordinates gene expression across different tissues to produce carotenoid-based red ornamentation. Molecular Biology and Evolution 40: msad056. https://doi.org/10.1093/molbev/msad056.

Kijimoto, T., and A. P. Moczek. 2016. Hedgehog signalling enables nutrition-responsive inhibition of an alternative morph in a polyphenic beetle. Proceedings of the National Academy of Sciences of the USA 113:5982–5987. https://doi.org/10.1073/pnas.1601505113.

Kijimoto, T., A. P. Moczek, and J. Andrews. 2012. Diversification of *doublesex* function underlies morph-, sex-, and species-specific development of beetle horns. Proceedings of the National Academy of Sciences of the USA 109:20526–20531. https://doi.org/10.1073/pnas.1118589109.

Kijimoto, T., E. C. Snell-Rood, M. H. Pespeni, G. Rocha, K. Kafadar, and A. P. Moczek. 2014. The nutritionally responsive transcriptome of the polyphenic beetle *Onthophagus taurus* and the importance of sexual dimorphism and body region. Proceedings of the Royal Society B 281:20142084. https://doi.org/10.1098/rspb.2014.2084.

Kitano, J., A. Ishikawa, and S. C. Lema. 2014. Integrated genomics approaches in evolutionary and ecological endocrinology. Pages 299–319 *in* C. R. Landry and N. Aubin-Horth, eds. Ecological genomics. Advances in Experimental Medicine and Biology 781. Springer, Dordrecht.

Kitano, J., Y. Kawagishi, S. Mori, C. Peichel, T. Makino, M. Kawata, and M. Kusakabe. 2011. Divergence in sex steroid hormone signaling between sympatric species of Japanese threespine stickleback. PLoS ONE 6:e29253. https://doi.org/10.1371/journal.pone.0029253.

Lagunas, N., J. M. Fernández-García, N. Blanco, A. Ballesta, B. Carrillo, M.-A. Arevalo, P. Collado, H. Pinos, and D. Grassi. 2022. Organizational effects of estrogens and androgens on estrogen and androgen receptor expression in pituitary and adrenal glands in adult male and female rates. Frontiers in Neuroanatomy 16:902218. https://doi.org/10.3389/fnana.2022.902218.

Lahaye, S. E. P., M. Eens, V. M. Darras, and R. Pinxten. 2012. Testosterone stimulates the expression of male-typical socio-sexual and song behaviors in female budgerigars (*Melopsittacus undulatus*): an experimental study. General and Comparative Endocrinology 178:82–88. https://doi.org/10.1016/j.ygcen.2012.04.021.

———. 2014. Bare-part color in female budgerigars changes from brown to structural blue following testosterone treatment but is not strongly masculinized. PLoS ONE 9:e86849. https://doi.org/10.1371/journal.pone.0086849.

Leaché, A. D., B. L. Banbury, C. W. Linkem, and A. Nieto-Montes de Oca. 2016. Phylogenomics of a rapid radiation: is chromosomal evolution linked to increased diversification in North American spiny lizards (genus *Sceloporus*)? BMC Evolutionary Biology 16:63. https://doi.org/10.1186/s12862-016-0628-x.

Ledón-Rettig, C. C., and A. P. Moczek. 2016. The transcriptomic basis of tissue- and nutrition-dependent sexual dimorphism in the beetle *Onthophagus taurus*. Ecology and Evolution 6:1601–1613. https://doi.org/10.1002/ece3.1933.

Ledón-Rettig, C. C., E. E. Zattara, and A. P. Moczek. 2017. Asymmetric interactions between *doublesex* and tissue- and sex-specific target genes mediate sexual dimorphism in beetles. Nature Communications 8:14593. https://doi.org/10.1038/ncomms14593.

Lema, S. C. 2014. Hormones and phenotypic plasticity in an ecological context: linking physiological mechanisms to evolutionary processes. Integrative and Comparative Biology 54:850–863. https://doi.org/10.1093/icb/icu019.

Liao, Y., G. K. Smyth, and W. Shi. 2013. The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. Nucleic Acid Research 41:e108. https://doi.org/10.1093/nar/gkt214.

———. 2014. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30:923–930. https://doi.org/10.1093/bioinformatics/btt656.

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

Madison, F. N., M. L. Rouse Jr., J. Balthazart, and G. F. Ball. 2015. Revising song behavior phenotype: testosterone driven induction of singing and measures of song quality in adult male and female canaries (*Serinus canaria*). General and Comparative Endocrinology 215:61–75. https://doi.org/10.1016/j.ygcen.2014.09.008.

Mangiamele, L. A., and M. J. Fuxjager. 2018. Insight into the neuroendocrine basis of signal evolution: a case study in foot-flagging frogs. Journal of Comparative Physiology A 204:61–70. https://doi.org/10.1007/s00359-017-1218-0.

Mangiamele, L. A., M. J. Fuxjager, E. R. Schuppe, R. S. Taylor, W. Hödl, and D. Preininger. 2016. Increased androgenic sensitivity in the hind limb muscular system marks the evolution of a derived gestural display. Proceedings of the National Academy of Sciences of the USA 113:5664–5669. https://doi.org/10.1073/pnas.1603329113.

Manoli, D., and J. Tollkuhn. 2018. Gene regulatory mechanisms underlying sex differences in brain development and psychiatric disease. Annals of the New York Academy of Sciences 1420:26–45. https://doi.org/10.1111/nyas.13564.

Mauro, A. A., and C. K. Ghalambor. 2020. Trade-offs, pleiotropy, and shared molecular pathways: a unified view of constraints on adaptation. Integrative and Comparative Biology 60:332–347. https://doi.org/10.1093/icb/icaa056.

McAbee, M. D., and L. L. DonCarlos. 1998. Ontogeny of region-specific sex differences in androgen receptor messenger ribonucleic acid expression in the rat forebrain. Endocrinology 139:1738–1745. https://doi.org/10.1210/endo.139.4.5940.

McCarthy, M. M. 2016. Multifaceted origins of sex differences in the brain. Philosophical Transactions of the Royal Society B 371:20150106. https://doi.org/10.1098/rstb.2015.0106.

McCarthy, M. M., C. L. Wright, and J. M. Schwarz. 2009. New tricks by an old dogma: mechanisms of the organizational/activational hypothesis of steroid-mediated sexual differentiation of brain and behavior. Hormones and Behavior 55:655–665. https://doi.org/10.1016/j.yhbeh.2009.02.012.

McGlothlin, J. W., and E. D. Ketterson. 2008. Hormone-mediated suites as adaptations and evolutionary constraints. Philosophical Transactions of the Royal Society B 363:1611–1620. https://doi.org/10.1098/rstb.2007.0002.

———. 2016. Hormonal pleiotropy and the evolution of correlated traits. Pages 100–119 *in* E. D. Ketterson and J. W. Atwell, eds. Snowbird: integrative biology and evolutionary diversity in the junco. University of Chicago Press, Chicago.

McGlothlin, J. W., M. E. Kobiela, H. V. Wright, J. J. Kolbe, J. B. Losos, and E. D. Brodie III. 2022 Conservation and convergence of genetic architecture in the adaptive radiation of *Anolis* lizards. American Naturalist 200:207–220. https://doi.org/10.1086/721091.

Mittal, K., P. F. P. Henry, R. S. Cornman, C. Maddox, N. Basu, and N. K. Karouna-Renier. 2021. Sex- and developmental stage-related differences in the hepatic transcriptome of Japanese quail (*Coturnix japonica*) exposed to 17β-trenbolone. Environmental Toxicology and Chemistry 40:2559–2570. https://doi.org/10.1002/etc.5143.

Neubert da Silva, G., T. Z. Curi, S. E. L. Tolouei, M. T. Passoni, G. B. S. Hey, R. M. Romano, A. J. Martino-Andrade, and P. R. Dalsenter. 2019. Effects of diisopentyl phthalate exposure during gestation and lactation on hormone-dependent behaviours and hormone receptor expression in rats. Journal of Neuroendocrinology 31:e12816.

Newhouse, D. J., and B. J. Vernasco. 2020. Developing a transcriptomic framework for testing testosterone-mediated handicap hypotheses. General and Comparative Endocrinology 298:113577. https://doi.org/10.1016/j.ygcen.2020.113577.

Oliva, M., M. Muñoz-Aguirre, S. Kim-Hellmuth, V. Wucher, A. D. H. Gewirtz, D. J. Cotter, P. Parsana, et al. 2020. The impact of sex on gene expression across human tissues. Science 369:eaba3066. https://doi.org/10.1126/science.aba3066.

Ossip-Drahos, A. G., J. R. Oyola Morales, C. Vital-García, J. J. Zúñiga-Vega, D. K. Hews, and E. P. Martins. 2016. Shaping communicative colour signals over evolutionary time. Royal Society Open Science 3:160728. https://doi.org/10.1098/rsos.160728.

Partridge, C., C. M. C. Rodgers, R. Knapp, and B. D. Neff. 2015. Androgen effects on immune gene expression during parental care in bluegill sunfish (*Lepomis macrochirus*). Canadian Journal of Zoology 93:9–13. https://doi.org/10.1139/cjz-2014-0157.

Peterson, M. P., K. A. Rosvall, J.-H. Choi, C. Ziegenfus, H. Tang, J. K. Colbourne, and E. D. Ketterson. 2013. Testosterone affects neural gene expression differently in male and female juncos: a role for hormones mediating sexual dimorphism and conflict. PLoS ONE 8:e61784. https://doi.org/10.1371/journal.pone.0061784.

Peterson, M. P., K. A. Rosvall, C. A. Taylor, J. A. Lopez, J.-H. Choi, C. Ziegenfus, H. Tang, J. K. Colbourne, and E. D. Ketterson. 2014. Potential for sexual conflict assessed via testosterone-mediated transcriptional changes in liver and muscle of a songbird. Journal of Experimental Biology 217:507–517. https://doi.org/10.1242/jeb.089813.

Phipson, B., S. Lee, I. J. Majewski, E. S. Alexander, and G. K. Smyth. 2016. Robust hyperparameter estimation protects against hypervariable genes and improves power to detect differential expression. Annals of Applied Statistics 10:946–963. https://doi.org/10.1214/16-AOAS920.

Phoenix, C. H., R. W. Goy, A. A. Gerall, and W. C. Young. 1959. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology 65:369–382. https://doi.org/10.1210/endo-65-3-369.

Prud'homme, B., N. Gompel, and S. B. Carroll. 2007. Emerging principles of regulatory evolution. Proceedings of the National

Academy of Sciences of the USA 104:8605–8612. https://doi.org/10.1073/pnas.0700488104.

Quinn, V. S., and D. K. Hews. 2003. Positive relationship between abdominal coloration and dermal melanin density in phrynosomatid lizards. Copeia 2003:858–864. https://doi.org/10.1643/h202-116.1.

Rabinowitz, A. H., and S. A. Vokes. 2012. Integration of the transcriptional networks regulating limb morphogenesis. Developmental Biology 368:165–180. https://doi.org/10.1016/j.ydbio.2012.05.035.

R Core Team. 2022. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna.

Reynkens, T. 2018. rospca: robust sparse PCA using ROSPCA algorithm. R package version 1.0.4.

Rhen, T., J. Ross, and D. Crews. 1999. Effects of testosterone on sexual behavior and morphology in adult female leopard geckos, *Eublepharis macularius*. Hormones and Behavior 36:119–128. https://doi.org/10.1006/hbeh.1999.1530.

Rinn, J. L., and M. Snyder. 2005. Sexual dimorphism in mammalian gene expression. Trends in Genetics 21:298–305. https://doi.org/10.1016/j.tig.2005.03.005.

Robinson, C. D., M. D. Hale, C. Cox, H. John-Alder, and R Cox. 2024. Data from: Effects of testosterone on gene expression are concordant between sexes but divergent across species of *Sceloporus* lizards. American Naturalist, Dryad Digital Repository, https://doi.org/10.5061/dryad.fj6q57432.

Robinson, C. D., M. D. Hale, T. N. Wittman, C. L. Cox, H. B. John-Alder, and R. M. Cox. 2023. Species differences in hormonally mediated gene expression underlie the evolutionary loss of sexually dimorphic coloration in *Sceloporus* lizards. Journal of Heredity 114:637–653. https://doi.org/10.1093/jhered/esad046.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140. https://doi.org/10.1093/bioinformatics/btp616.

Roff, D. A., and D. J. Fairbairn. 2007. The evolution of trade-offs: where are we? Journal of Evolutionary Biology 20:433–447. https://doi.org/10.1111/j.1420-9101.2006.01255.x.

Romero, I. G., I. Ruvinsky, and Y. Gilad. 2012. Comparative studies of gene expression and the evolution of gene regulation. Nature Reviews Genetics 13:505–516. https://doi.org/10.1038/nrg3229.

Rosa-Molinar, E., B. Fritzsch, and S. E. Hendricks. 1996. Organizational-activational concept revisited: sexual differentiation in an atherinomorph teleost. Hormones and Behavior 30:563–575. https://doi.org/10.1006/hbeh.1996.0059.

Rose, E. M., C. M. Haakenson, K. Stennette, A. Patel, S. Gaind, B. D. Shank, F. N. Madison, and G. F. Ball. 2022. Neuroendocrine and behavioral response to testosterone-induced female song in canaries (*Serinus canaria*). Physiology and Behavior 250:113782. https://doi.org/10.1016/j.physbeh.2022.113782.

Rosvall, K. A. 2022. Evolutionary endocrinology and the problem of Darwin's tangled bank. Hormones and Behavior 146:105246. https://doi.org/10.1016/j.yhbeh.2022.105246.

Rosvall, K. A., C. M. Bergeon Burns, S. P. Jayaratna, E. K. Dossey, and E. D. Ketterson. 2016*b*. Gonads and the evolution of hormone phenotypes. Integrative and Comparative Biology 56:225–234. https://doi.org/10.1093/icb/icw050.

Rosvall, K. A., C. M. Bergeon Burns, S. P. Jayaratna, and E. D. Ketterson. 2016*a*. Divergence along the gonadal steroidogenic pathway: implications for hormone-mediated phenotypic evolution. Hormones and Behavior 84:1–8. https://doi.org/10.1016/j.yhbeh.2016.05.015.

Sackton, T. B., P. Grayson, A. Cloutier, Z. Hu, J. S. Liu, N. E. Wheeler, P. P. Gardner, et al. 2019. Convergent regulatory evolution and loss of flight in paleognathous birds. Science 364:74–78. https://doi.org/10.1126/science.aat7244.

Schwab, D. B., K. D. Newsom, and A. P. Moczek. 2020. Serotonin signalling suppresses the nutrition-responsive induction of an alternate male morph in horn polyphenic beetles. Journal of Experimental Zoology A 333:660–669. https://doi.org/10.1002/jez.2413.

Schweitzer, C., M. H. Goldstein, N. J. Place, and E. Adkins-Regan. 2013. Long-lasting and sex-specific consequences of elevated egg yolk testosterone for social behavior in Japanese quail. Hormones and Behavior 63:80–87. https://doi.org/10.1016/j.yhbeh.2012.10.011.

Snell-Rood, E. C., and A. P. Moczek. 2012. Insulin signalling as a mechanism underlying development plasticity: the role of FOXO in a nutritional polyphenisms. PLoS ONE 7:e34857. https://doi.org/10.1371/journal.pone.0034857.

Stearns, S. C. 1989. Trade-offs in life-history evolution. Functional Ecology 3:259–268. https://doi.org/10.2307/2389364.

Streisfeld, M. A., and M. D. Rausher. 2009. Genetic changes contributing to the parallel evolution of red floral pigmentation among *Ipomoea* species. New Phytologist 183:751–763. https://doi.org/10.1111/j.1469-8137.2009.02929.x.

Sullivan, D. A., R. V. Jensen, T. Suzuki, and S. M. Richards. 2009. Do sex steroids exert sex-specific and/or opposite effects on gene expression in lacrimal and meibomian glands? Molecular Vision 15:1553–1572. http://www.molvis.org/molvis/v15/a166.

Swanson, E. M., and B. Dantzer. 2014. Insulin-like growth factor-1 is associated with life-history variation across Mammalia. Proceedings of the Royal Society B 281:20132458. https://doi.org/10.1098/rspb.2013.2458.

Tobias, M. L., M. L. Marin, and D. B. Kelley. 1991. Temporal constraints on androgen directed laryngeal masculinization in *Xenopus laevis*. Developmental Biology 147:260–270. https://doi.org/10.1016/S0012-1606(05)80023-5.

Tsuboi, M., W. van der Bijl, B. T. Kopperud, J Erritzøe, K. L. Voje, A. Kotrschal, K. E. Yopak, S. P. Collin, A. N. Iwaniuk, and N. Kolm. 2018. Breakdown of brain-body allometry and the encephalization of birds and mammals. Nature Ecology and Evolution 2:1492–1500. https://doi.org/10.1038/s41559-018-0632-1.

van Nas, A., D. GuhaThakurta, S. S. Wang, N. Yehya, S. Horvath, B. Zhang, L. Ingram-Drake, et al. 2009. Elucidating the role of gonadal hormones in sexually dimorphic gene coexpression networks. Endocrinology 150:1235–1249. https://doi.org/10.1210/en.2008-0563.

Westfall, A. K., R. S. Telemeco, M. B. Grizante, D. S. Waits, A. D. Clark, D. Y. Simpson, R. L. Klabacka, et al. 2021. A chromosome-level genome assembly for the eastern fence lizard (*Sceloporus undulatus*), a reptile model for physiological and evolutionary ecology. GigaScience 10:giab066. https://doi.org/10.1093/gigascience/giab066.

Wiens, J. J. 1999. Phylogenetic evidence for multiple losses of a sexually selected character in phrynosomatid lizards. Proceedings of the Royal Society B 266:1529–1535. https://doi.org/10.1098/rspb.1999.0811.

Wittman, T. N., C. D. Robinson, J. W. McGlothlin, and R. M. Cox. 2021. Hormonal pleiotropy structures genetic covariance. Evolution Letters 5:397–407. https://doi.org/10.1002/evl3.240.

References Cited Only in the Online Enhancements

Ashburner, M., C. A. Ball, J. A. Blake, D. Botsteain, H. Butler, J. M. Cherry, A. P. Davis, et al. 2000. Gene ontology: tool for the unification of biology. Nature Genetics 25:9.

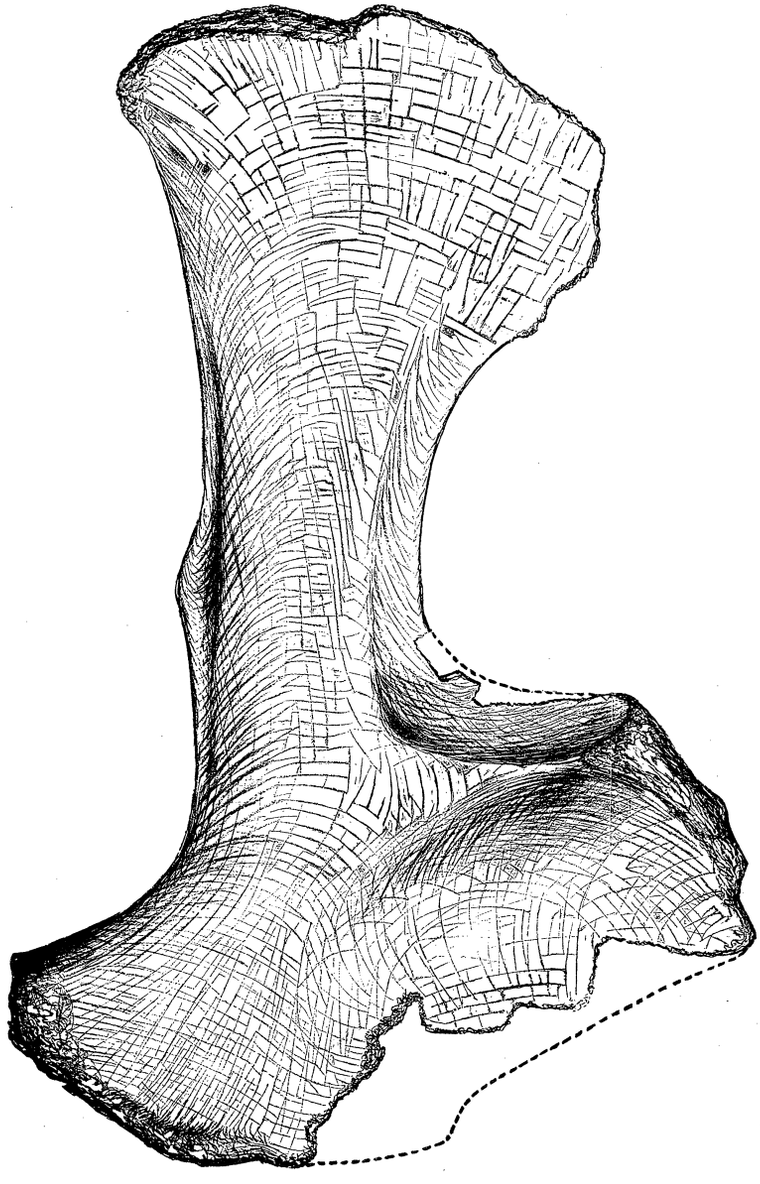
Gene Ontology Consortium. 2021. The gene ontology resource: enriching a GOld mine. Nucleic Acid Research 49:D325–D334.

Hews, D. K., E. Hara, and M. C. Anderson. 2012. Sex and species differences in plasma testosterone and in counts of androgen receptor-positive cells in key brain regions of *Sceloporus* lizard species that differ in aggression. General and Comparative Endocrinology 176:493–499.

John-Alder, H. B., R. M. Cox, G. J. Haenel, and L. C. Smith. 2009. Hormones, performance and fitness: natural history and endocrine experiments on a lizard (*Sceloporus undulatus*). Integrative and Comparative Biology 49:393–407.

Smith, L. C., and H. B. John-Alder. 1999. Seasonal specificity of hormonal, behavioral, and coloration responses to within- and between-sex encounters in male lizards (*Sceloporus undulatus*). Hormones and Behavior 36:39–52.

Associate Editor: Alison M. Bell
Editor: Erol Akçay



"The right scapula of *Camarasaurus supremus*, external view." From "On the Saurians Recently Discovered in the Dakota Beds of Colorado" by E. D. Cope (*The American Naturalist*, 1878, 12:71–85).