

SYNTHESIS

The Ancestral Modulation Hypothesis: Predicting Mechanistic Control of Sexually Heteromorphic Traits Using Evolutionary History

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ABSTRACT: Across the animal kingdom there are myriad forms within a sex across, and even within, species, rendering concepts of universal sex traits moot. The mechanisms that regulate the development of these trait differences are varied, although in vertebrates, common pathways involve gonadal steroid hormones. Gonadal steroids are often associated with heteromorphic trait development, where the steroid found at higher circulating levels is the one involved in trait development for that sex. Occasionally, there are situations in which a gonadal steroid associated with heteromorphic trait development in one sex is involved in heteromorphic or monomorphic trait development in another sex. We propose a verbal hypothesis, the ancestral modulation hypothesis (AMH), that uses the evolutionary history of the trait—particularly which sex ancestrally possessed higher trait values—to predict the regulatory pathway that governs trait expression. The AMH predicts that the genomic architecture appears first to resolve sexual conflict in an initially monomorphic trait. This architecture takes advantage of existing sex-biased signals, the gonadal steroid pathway, to generate trait heteromorphism. In cases where the other sex experiences evolutionary pressure for the new phenotype, that sex will co-opt the existing architecture by altering its signal to match that of the original high-trait-value sex. We describe the integrated levels needed to produce this pattern and what the expected outcomes will be given the evolutionary history of the trait. We present this framework as a testable hypothesis for the scientific community to investigate and to create further engagement and analysis of both ultimate and proximate approaches to sexual heteromorphism.

Keywords: sexual dimorphism, evolution, hormones, *cis*-regulatory elements, transcription, trait regulation.

Introduction

Across the animal kingdom there is a wide range of traits in which the phenotypic value correlates with the sex of its bearer. Such traits are said to be sexually dimorphic and can result from multiple modes of selection, including sexual selection, intersexual resource competition, and niche partitioning (Hedrick and Temeles 1989). Moving forward in this article, we will instead use the term “heteromorphic,” as dimorphic implies a lack of trait value overlap and there can be multiple modes in trait value within and across sexes. Traditionally, some traits have been viewed as “male” while others have been viewed as “female,” due to Darwin’s writings (Darwin 1871) and to the use of anisogamy as the primary explanatory driver of sex differences (Trivers 1972). While these early writings acknowledged the existence of species that bucked these trends, a full appreciation for the diversity of and selection on traits within and across sexes and species would come later (Gowaty 1982; Hrdy 1986, 2013; Clutton-Brock 2009; Clutton-Brock and Huchard 2013). Evolution has produced some startling examples counter to earlier conceptions of sexually heteromorphic phenotypes. The most prominent clades that contain species contrary to these notions include syngnathid fishes (pipefishes, seahorses, and sea dragons; Berglund et al. 1986; Jones et al. 2001; Flanagan et al. 2014), dendrobatid frogs (poison dart frogs; Weygoldt 1987), and charadriiform birds (e.g., jacanas, buttonquails, and sandpipers; Höhn and Cheng 1967; Fivizzani and Oring 1986; Emlen and Wrege 2004; Voigt 2016). In each of these clades, there are species in which females possess larger ornaments and weaponry, as well as more aggression and courtship behavior, compared with males, whereas males can primarily engage in mate choice

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and brood care. More extreme examples of stereotype-breaking traits include the intromittent reproductive organ of a female arthropod (Yoshizawa et al. 2018) and the pseudopenis of spotted hyenas (Matthews 1939; Glickman et al. 1987). As science has moved forward, the concept of universal male and female traits has been discarded with a view toward the appreciation of multimodality in sex-biased traits (McLaughlin et al. 2023), although evolutionary patterns are still present (Janicke et al. 2016; Winkler et al. 2021).

The evolution of mechanistic pathways that govern sexually heteromorphic traits continues to be an active research area in evolutionary biology. Underlying these heteromorphic features are the genes responsible for shaping their formation, implicating a genomic architecture that produces differential expression of genes between the sexes, termed “sex-biased gene expression” (Ellegren and Parsch 2007; Pointer et al. 2013; Harrison et al. 2015; Mank and Rideout 2021). Starting with Lande (1980), evolutionary biologists have continued to uncover the mechanisms that allow for heteromorphic features to evolve despite intralocus sexual conflict, defined as an allele at a locus having separate fitness effects depending on the bearing sex (Connallon and Clark 2014). Initially, heterogametic chromosomes were thought to be a primary resolution to intralocus sexual conflict (Rice 1984); however, many animals only have autosomal chromosomes, and the effects of heterogametic chromosomes are less influential than previously thought (Bonduriansky and Chenoweth 2009; Mank 2009, 2017; Stewart et al. 2010). A wider array of possible resolutions has been put forward (Bonduriansky and Chenoweth 2009; Pennell and Morrow 2013; Mank 2017), but one feature continuously reoccurs in vertebrates in both theoretical models and empirical observations: direct genomic regulation via sex-biased signals (Wray 2007; Wittkopp and Kalay 2012; Wilkinson et al. 2015). These sex-biased signals are most often sex steroids in vertebrates that contribute to the organization and activation of sexually heteromorphic traits and can further break down genetic correlations (Ketterson et al. 2005; McGlothlin and Ketterson 2008; Cox et al. 2017; Wittman et al. 2021). We will refer to sex steroids as “gonadal hormones,” since not all functionality is sex related and the gonads are the primary site of production establishing sexual heteromorphism in circulating levels. The mechanistic control of sex-biased gene expression via gonadal hormonal signaling, while not the only mechanism of sexual heteromorphism, is the focus of our article, since it is both an important aspect of sexual heteromorphism and, as shown with numerous examples in this text, widely prevalent in vertebrate systems (Bardin and Catterall 1981; Owens and Short 1995; Goymann and Wingfield 2014).

With the expectation that gonadal steroids are associated with higher circulating levels in one sex and therefore

should regulate heteromorphic traits for that sex, the times where these situations do not occur merit some explanation. An ornament present in females might be expected to be regulated by estrogens, as is the case for iridescent bands in Gulf pipefish (Partridge et al. 2010), but there are examples of heteromorphic ornamentation with higher trait values in females being testosterone mediated, such as for the dark plumage on the breast in the barred buttonquail (Muck and Goymann 2011). Another situation that can occur is when females secondarily acquire ornamental trait values present in males, making the sexes monomorphic for that trait. An example of this is seen in populations of white-shouldered fairywrens, where the white-shouldered ornaments are present in females for some populations and those ornaments are testosterone mediated in both sexes (Enbody et al. 2022). Why a sex might use a low-circulating gonadal steroid to regulate a heteromorphic trait (e.g., estrogen control in males) or why a monomorphic trait might be regulated by a heteromorphic gonadal steroid does not fit into most descriptions of resolutions to intralocus genomic conflict.

The premise of much of the research into sexually heteromorphic traits is the assumption that the ancestral condition of the trait was monomorphic. Over evolutionary time, however, a trait that may have been favorable for one sex—and hence heteromorphic—may now become favorable in the other sex, leading to renewed monomorphism or even a reversed sexual heteromorphism. West-Eberhard (2003) termed the traits resulting from these evolutionary events “cross-sexual transfers,” which she defined as “discrete traits that are expressed exclusively in one sex in an ancestral species [that] appear in the opposite sex of descendants” (p. 260). West-Eberhard laid out several features to identify a trait as a putative product of cross-sexual transfer, but only one describes a mechanistic pattern by testing gonadal hormonal regulation in causing trait values in one sex to match those in the other sex (box 1). There are many examples of exogenous and endogenous gonadal hormones influencing traits in the sex where those hormones occur at lower circulating levels, yet there is no solid evidence that the sexes can flip the dominant gonadal hormone to that of the other sex (Eens and Pinxten 2000). We therefore see a need to expand on the descriptions of mechanistic trait regulation to create a more robust test of cross-sexual transfer.

Given the diverse examples of stereotype-breaking species previously mentioned, there is a question as to whether these traits are the product of cross-sexual transfer or independently evolved in the observed sex. While the frequency of cross-sexual transfers is unknown, both West-Eberhard and we believe that they are more frequent than currently appreciated. Cross-sexual transfer as initially envisioned by West-Eberhard was rooted in plasticity

Box 1: Suggested indicators of putative cross-sexual transfer taken from West-Eberhard (2003)

- Trait composed of characteristics more typical of the other sex in the same or related species.
- Trait present in a subset of one sex that resembles the other sex in a heteromorphic species.
- Trait present in both sexes but phylogenetic information suggests a heteromorphic ancestor (secondary monomorphism).
- Trait is more complete in one sex, which is the ancestrally bearing sex, compared with the other sex, the secondary sex.
- Trait is maladaptive in one sex, the secondary sex, due to interference with typical functions of that sex, but not the other sex, the ancestral sex.
- Traits in one sex can be induced via gonadal hormones found at higher circulating levels in the other sex.

(2003), where a small shift due to the environment or expression can produce new trait values in one sex, which selection then favors to shift the population toward that sensitivity and trait value. A trait undergoing or that is the product of cross-sexual transfer need not have a complex evolutionary process via multiple novel genetic structures to produce the trait but rather a few changes to co-opt existing genomic structures via sensitivity to hormonal signals through alteration in a few genes, an alteration in environmental input, or a combination of the two. West-Eberhard acknowledged the importance of both developmental organizing effects and activational effects on cross-sexual transfer, but for the purposes of this article we do not seek to describe the different effects these could have on cross-sexual transfers.

Here, we present a hypothesis that evolutionary history can predict the mechanistic patterns observed in trait formation. We further describe each level of trait regulation

and what the expected pattern should be given the evolutionary history and highlight examples that may suggest whether the trait is a product of a novel sexual heteromorphism or a cross-sexual transfer. Last, we offer ways to investigate our framework and present some different scenarios and how our hypothesis might explain the observed patterns.

The Ancestral Modulation Hypothesis

We describe a conceptual framework based on current evidence, the ancestral modulation hypothesis (AMH), as a causative link between cross-sexual transfer of a given trait and the current mechanistic pathways that govern the trait's expression. The AMH lays out two mechanistic patterns (fig. 1) spanning multiple levels of regulation (signal: hormone titers, local synthesis, and expression of receptors; architecture: regulatory elements and transcriptional

Ancestral Modulation Hypothesis	
• If the regulatory mechanism for a sexually heteromorphic trait matches a Type I pattern, the trait emerged from a monomorphic ancestor • If the regulatory mechanism for a sexually heteromorphic or monomorphic trait matches a Type II pattern, the trait emerged from cross-sexual transfer	
Type I	Type II
A sexually heteromorphic trait is responsive to the gonadal hormone with higher levels due to: • Typical circulating ratios • Local sensitivity to high level hormones through receptors • Local synthesis of a more potent gonadal hormone • Accessing genes through HREs associated with high level hormones	A trait in one sex (either monomorphic or sexually heteromorphic) is responsive to the gonadal hormone with lower levels due to: • Altered circulating ratios • Local sensitivity to low level hormones through receptors • Local synthesis of gonadal hormones to ones found at higher levels in another sex • Accessing genes through HREs associated with low level hormones

Figure 1: Definition for the ancestral modulation hypothesis and associated hormonal control patterns. For each pattern, multiple mechanisms may evolve to access associated hormone response elements (HREs) to alter gene expression.

patterns) that would indicate whether cross-sexual transfer can explain the currently observed trait or whether the trait emerged *de novo*. The AMH can be thought of as a time series through the evolutionary past of a given trait (fig. 2, 3). First, a monomorphic trait undergoes changes to genomic architecture using and/or enhancing existing sex-bias signaling to become heteromorphic (type I). Second, the now heteromorphic trait undergoes alterations to sex-bias signaling in one sex while keeping the genomic architecture of the heteromorphic condition to become a trait produced by cross-sexual transfer (type II). We define the genomic portion—particularly the response elements, the portion of the genome bound by the nuclear hormone receptor to activate/inhibit transcription—as the bridge between genomic architecture and signal because genomic architecture can be altered only intergenerationally while the signal (receptors, enzymes, hormones) can be plastic in its environmental response (Westrick et al. 2022). This broad description of the AMH could have far-reaching application across all kinds of organisms and multiple levels of mechanisms underlying heteromorphism. The framework of the AMH connects the regulatory phenomena of

heteromorphic trait formation through architecture and signal to the evolutionary history of which sex(es) had higher trait values through time. Most of the evidence is found in vertebrates and the sex-signaling process of hormones, specifically gonadal steroid hormones. Indeed, numerous studies have demonstrated that multiple gonadal steroids are active in trait formation within a sex (Brown 1985; Cooper and Crews 1987; Specker and Kishida 2000), such as bird song (Alward et al. 2018), so much so that despite the heteromorphic levels of gonadal hormones it is often inappropriate to consider them exclusively male or female, much like other phenotypic traits discussed above. We therefore present a specific and narrower focus of the AMH that is currently tractable with the intention that the generalizability of the AMH across other sex signals, architectures, and taxa will later be tested. The purpose of the AMH is to identify the mechanistic pattern through an integrated approach and to predict expected mechanisms based on evolutionary history or vice versa. A secondary purpose of the AMH is to provide a broad framework for describing and discussing mechanistic patterns and the evolution of sex-biased regulation. The AMH does not

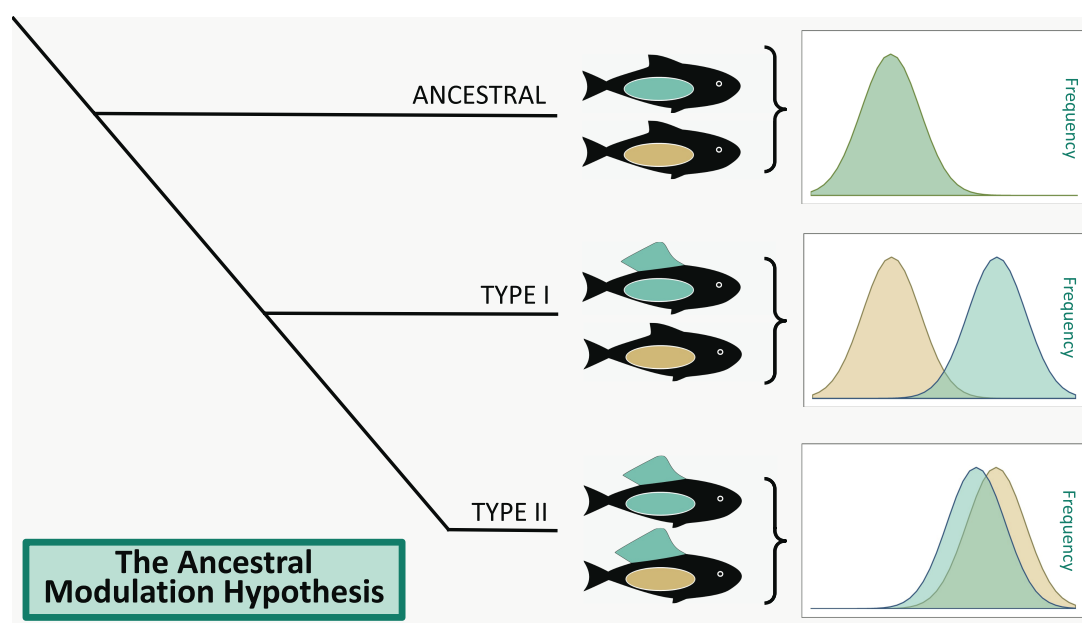


Figure 2: Proposed evolutionary history of a trait (e.g., dorsal fin size and color) according to the framework of the ancestral modulation hypothesis. The trait value was monomorphic in an ancestral population, yet heteromorphic signals via gonadal hormones (green and beige) are present. Some selective force favored larger trait values in one sex (type I), so new transcriptomic patterns and hormone response elements (HREs) associated with the higher circulating gonadal hormone for that sex (e.g., green) emerged. Additional enhancements to the heteromorphic signal may evolve that cause further differentiation of sexually heteromorphic trait values. Following these changes, a new selective pressure on the low trait value sex to move toward the higher trait values either within a population or among species (type II). The sex in which these high trait values (beige) is now favored already has the genomic architecture (HREs and transcriptomic genes) to reach these trait values but has low levels of the gonadal hormone associated with the trait. The HREs do not change, but the co-opting sex (beige) evolves mechanisms to enhance to the signal of the low-circulating gonadal heteromorphic hormone.

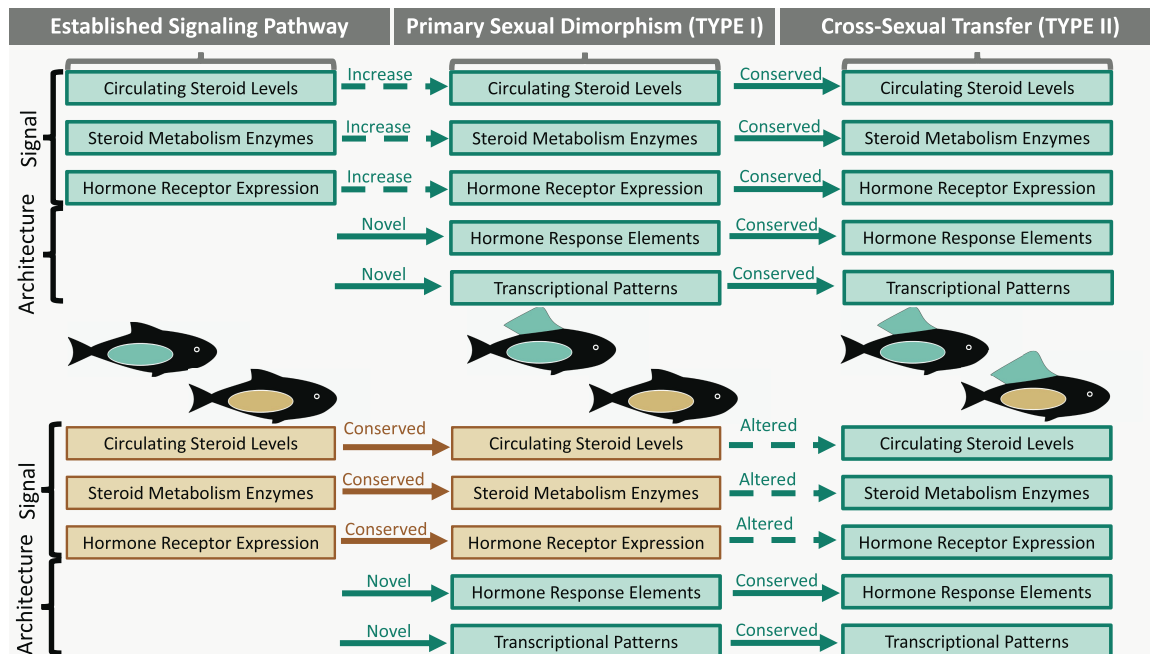


Figure 3: Diagram of mechanistic controls and conditions to test when investigating the ancestral modulation hypothesis. The general pattern is described in figure 2. For the ancestral (*left*), type I (*middle*), and type II (*right*) species in both sexes (green, *top*; beige, *bottom*) there are five levels to measure and compare: circulating gonadal steroid levels, gonadal steroid metabolism enzyme gene expression, gonadal hormone receptor expression (the previous three are the “signal”), transcriptomic patterns associated with the trait, and gonadal hormone response element changes associated with the gene set identified in the transcriptomic pattern (the previous two are the “architecture”). Transitions that must happen are shown as solid lines, and those that could happen are shown as dashed lines. Comparing ancestral and type I will see a change in architecture measures (increase in hormone response elements and novel transcripts) in both sexes since they share the genome. The architecture should favor expression in one sex (green). The trait-bearing sex in type I (green) could have changes to increase its signal in any three measures by increasing those values compared with the same sex in the ancestral species, but these changes are not necessary, like those in the genomic architecture. Comparing type I to type II focuses on the nonbearing sex in type I (beige). In type II, that sex (beige) now has trait values similar to the other sex from type I (green), which may or may not have those same trait values in type II. The architecture remains unchanged and biased toward the original bearing sex (green). The other sex (beige) in type II alters its signal to respond more strongly to the heteromorphic hormone it has at low levels (green) but is necessary to access the architecture. Any one or more of the three measures for signal must change for that sex.

predict or describe what evolutionary forces may drive sexual heteromorphism (sexual selection, sexual antagonism, niche partitioning, etc.), only what the possible outcomes are when they occur. We therefore present this idea to the scientific community for discussion and evaluation for possible refinement or revision of the AMH based on current and future research.

The AMH describes multiple integrated levels of mechanistic control for a sexually heteromorphic trait from the global gonadal hormonal signals to the genomic architecture. The importance of considering integrated levels has been previously discussed and studied (Hau 2007; Ketterson et al. 2009), but through the AMH we seek to make predictions about what changes could happen at each level and how those are shaped by evolutionary history. In vertebrates, gonadal hormones shape many sexually heteromorphic traits, such as ornamentation (Bartos et al. 2012; Lindsay et al. 2016; Anderson et al. 2020), courtship

(Gonçalves et al. 2014; Ghosal and Sorensen 2016; Rose et al. 2022a), aggression (Hirschenhauser et al. 2004; Pärn et al. 2008; Muller 2017), and brood care (Johns and Liley 1970; Siegel and Rosenblatt 1975; Rodgers et al. 2006). Gonadal steroids are produced by differentiated gonads at different levels between the sexes, although other parts of the endocrine system also produce steroid hormones for signaling. These steroids can pass through the cell membrane where most are bound to their matched nuclear receptors, although some can be bound to membrane receptors used in alternate signaling pathways, such the G-protein-coupled receptor (GPCR) pathway (Kelly and Wagner 1999; Heinlein and Chang 2002; Thomas et al. 2006; Prossnitz et al. 2008). Prior to binding to a nuclear receptor, some hormones may be altered into different conformations via enzymes that can enhance or reduce the binding to the receptor or even change from one general steroid type (androgen) to another (estrogen; Baker

2004b; Baker et al. 2015). We focus on the genomic sites that are responsive to sex hormone receptors, the hormone response elements (HREs), which are short sequences in the genome (~15 bp) that are actively bound by hormone receptors (Geserick et al. 2005; Hahn 2007; Wittkopp and Kalay 2012). These HREs can influence transcriptional processes by up- or downregulating specific genes, leading to the differential expression observed between the sexes (Geserick et al. 2005; Wittkopp and Kalay 2012; Anderson and Jones 2022). Using the described hormone signaling pathway in vertebrates, the AMH presented here has two parts (signal and architecture) composed as follows: (1) the signal is composed of global hormone levels, biosynthetic enzymes altering cellular hormone levels, and receptor expression and (2) the architecture is composed of the HREs and transcriptomic patterns that shape the phenotype (fig. 3). While both biosynthetic enzymes and nuclear receptors are transcriptional responses, we categorize them as signals, since they are part of the cascade that generates the sex-biased response.

Type I traits have mechanisms that would be expected to emerge as a trait evolves from a monomorphic condition to a heteromorphic one and most genomic sexual conflict has been resolved. The mechanistic pattern would have new genomic and transcriptomic patterns compared with the ancestral condition, but the signaling cascade that results in sex-biased expression would be typical for other sexually heteromorphic features. To resolve sexual conflict, most evolutionary outcomes shape the genomic architecture of the trait to create a sex bias in expression patterns, as discussed earlier (Lande 1980; Bonduriansky and Chenoweth 2009; Connallon and Clark 2014; Mank 2017). Animals already have in place separate genomic architecture and signaling, which leads to gonadal differentiation (Hayes 1998; Devlin and Nagahama 2002; Pieau and Dorizzi 2004; Capel 2017) that reduces or resolves sexual antagonism for sex bias expression. Novel sexually heteromorphic characters evolve associations with these sex-specific developmental mechanisms and signaling pathways (Mank and Rideout 2021). We therefore expect a sexually heteromorphic trait that arose *de novo* from a monomorphic condition to gain sex-biased gene expression through new genomic features that bring those genes under the regulation of the existing sexually heteromorphic signaling cascade that is associated with other sexually heteromorphic features in that sex. In this case the ancestral signal for sex bias would be used, but novel architecture would evolve.

Type II traits are predicted to occur following cross-sexual transfer, where the trait itself changes little and instead the trait value distributions for the sexes are altered—in particular, the sex with ancestrally low trait values gains higher ones. Cross-sexual traits would have the same ge-

nomomic architecture as the ancestral sexually heteromorphic trait, but the signal would be altered in some way to access this architecture. The evolutionary hurdle for the one sex to express the underlying genes of a heteromorphic trait already biased for the other sex is that the architecture is already in place to generate the trait in the ancestrally bearing sex. There are two possible paths to resolve this issue: the first is to alter the architecture so both sexes or the ancestrally non-/low-expressing sex can use existing non-sex-biased signaling pathways, and the second is to increase the sex bias signal in the ancestrally non-/low-expressing sex to tap into the architecture already in place to produce trait values more similar to the ancestrally bearing sex.

One outcome that can appear to be cross-sexual transfer involves a reversion to the original monomorphic condition such that exaggerated features typical of heteromorphism are lost (West-Eberhard 2003). A striking example of this is found in fanged frogs, where secondary sex features such as thumbpads and advertisement call are lost in some groups, of which one species can produce these traits with an administration of a strong androgen (dihydrotestosterone [DHT]; Emerson et al. 1997). Such outcomes would not be considered cross-sexual transfer or a type II trait, as the sexual heteromorphism of the trait is lost instead of being expressed in the other sex—an alternative explanation that West-Eberhard put forward. Another outcome could be a change in the genomic architecture to allow for another signal to access the transcriptional pattern. Such changes would require the accumulation of HREs associated with another gonadal steroid or *cis*-regulatory elements associated with a monomorphic signal. While possible, the crux of cross-sexual transfer is in the ability of signals to be altered easily, inviting a more rapid evolutionary response by altering signal rather than the accumulation of response elements necessary to generate the trait value.

Instead of numerous genomic changes described above, a more parsimonious pathway is to alter signals to use existing sex-biased architecture, which has numerous examples, including altered hormone levels, biosynthetic enzymes, and hormone receptors that we describe throughout this article (fig. 4). The evolutionary change is an altering of the signaling cascade, such that a change in signal yielded phenotypic change while the genomic architecture remained mostly unaltered. West-Eberhard points out that the sensitivity of hormone titers to slight genetic and environmental changes makes them likely targets for shifts in sex-biased expression. We expand this to all aspects of the signal, and thus only a few genetic changes, or even none at all given strong environmental pressures, would lead to an individual bearing a sexually heteromorphic trait that has a genomic architecture structure that is responsive to the ancestrally bearing sex.

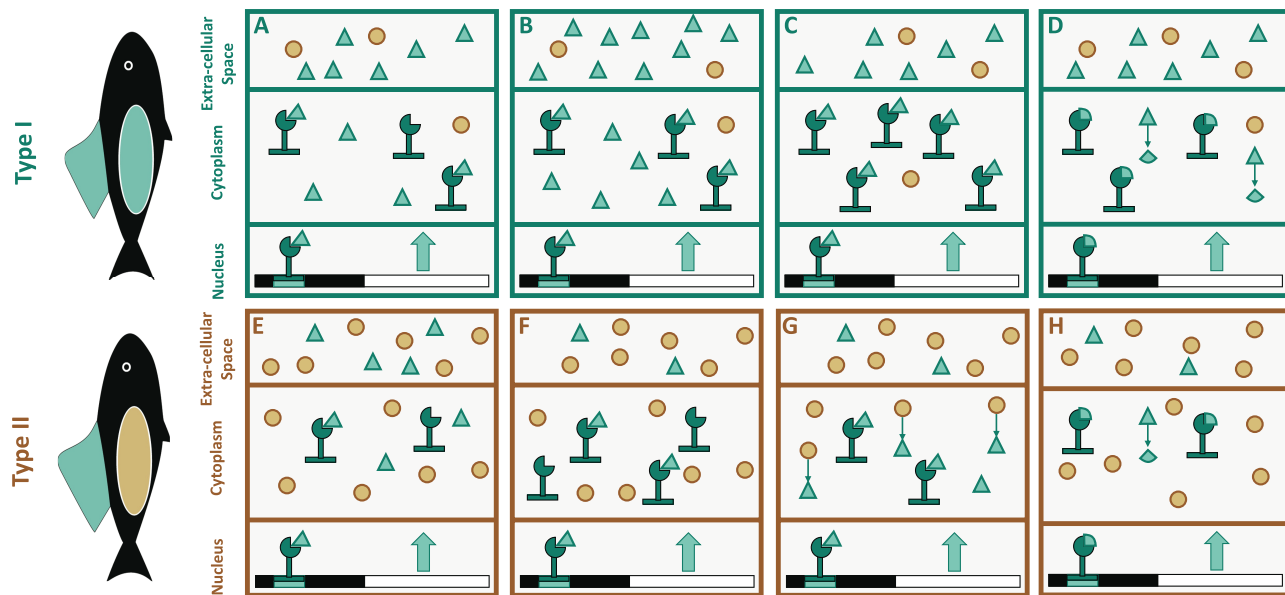


Figure 4: Diagram of different pathways to achieve transcriptomic response to generate a trait in a type I individual (green) that acquired the trait *de novo* and in a type II individual (beige) that acquired the trait via cross-sexual transfer. In a type I individual, the heteromorphic gonadal hormone present in the extracellular space at higher levels (green triangle) moves into the cytoplasm, where it is bound by associated nuclear receptors (T with three-quarters of a circle; A). The bound nuclear receptor (NR) binds to a hormone response element (HRE; small green bar) in the genome (black bar) to initiate transcriptions (white bar with arrow). A type I individual can increase the transcriptional response by increasing circulating levels of that hormone (B), increasing NR in the cytoplasm through increased NR expression (C), or converting the gonadal hormone to one with a higher affinity to the NR (green sector) via an increase in expression of biosynthetic enzymes (small green arrow; D). In a type II individual, the HREs and transcriptomic response are the same as the type I individual, which means the NR and hormone (green triangle) that binds it must also be the same, yet that hormone is not the higher circulating gonadal hormone (beige). A type II individual can increase the transcriptomic response by increasing circulating levels of the signal hormone (E), increasing NR in the cytoplasm through increased NR expression (F), converting one circulating hormone to the signal hormone via an increase in expression of biosynthetic enzymes (small arrow; G), or converting the gonadal hormone to one with a higher affinity to the NR (green sector) via an increase in expression of biosynthetic enzymes (small green arrow; H).

Below we describe the five mechanistic areas to be investigated when applying the AMH, either to confirm that the mechanisms match expectations of a known phylogeny or to use mechanisms to infer evolutionary history. We divide these areas into two groups, architecture and signal, which we have discussed previously, and provide a brief review of findings from the literature and how these findings can be interpreted within the AMH framework. Given the integrative nature of the AMH, no area on its own will support or reject the validity of the AMH nor confirm a type I or type II pattern; instead, each area should be considered in concert with others to paint the whole picture of the regulatory process. Last, we do not dwell on the definition of a trait, as they can manifest from a simple morphometric difference to an entire behavioral suite. We do suggest that traits be defined as narrowly as possible to limit confounding of interdependent traits and subsequent regulation. For example, bird song, as discussed above, has multiple gonadal hormones that shape it; however, song performance can be broken into parts as well as learning and acquisition. It would be more infor-

mative to address each one on its own to better track the evolutionary history of how each component might have developed more strongly from a monomorphic ancestor or from cross-sexual transfer that led to the overall trait observed. The degree to which the AMH applies equally to traits impacted by organizational versus activational effects of gonadal hormones remains to be tested, but until further evidence is collected it is not possible to draw a distinction.

Architecture

Transcriptome

Transcriptomes can illuminate which genes are putatively contributing to the sexual heteromorphism observed in the target species. A common practice is to expose individuals to exogenous hormones in an attempt to induce expression of the trait and identify shared expression in the target tissue where the naturally high-trait-value individual and the exogenously induced individual are

compared with the unexposed low-trait-value individual (Frankl-Vilches et al. 2015; Anderson et al. 2020). In cases where the influencing hormone is not known, a likely scenario when investigating AMH, trait-associated genes can be identified comparing across species. In manakins, species with a fast “snap” wing pattern in males have sets of genes that exhibit sex-biased expression concurrently with the developmental emergence of the snapping trait (Pease et al. 2022). These genes would be the targets of investigations at the other levels in the AMH, with the prediction that the snapping trait would be influenced by the androgen signal system (type I). Other approaches could look at changing expression levels with changing phenotypes. In wild turkeys, there is a continuum of secondary sex traits across males, where those with greater heteromorphism exhibit more differentially expressed genes (DEGs) than females (Pointer et al. 2013). In three species of finches, as more color heteromorphism emerges there are more DEGs, suggesting that expression levels are changing with trait expression, which is demonstrated with one gene (*BCO2*) shown to influence color expression (Gazda et al. 2020). These approaches would also generate a list of target genes for testing the AMH. The correlation of trait value and expression, though imperfect, makes for a useful tool (Lu et al. 2007) to identify target genes when information on ancestral condition is lacking. Importantly, transcriptomic data should be gathered from the tissues associated with trait formation—a simple task for structural phenotypes, but for behavioral phenotypes care must be taken to target the active neurological regions associated with the generated behavior. Furthermore, consideration of whether the trait has an organizational or activational basis is an important consideration, as the transcriptomic pattern will change with time and sampling should focus on when the trait emerges or is maintained.

The transcription profile for a given phenotype is a necessary component when investigating the AMH; however, the information gleaned will not directly provide evidence of a type I or type II trait. If a trait is sexually heteromorphic, it would be expected that the transcriptional profile of those tissues would have a high amount of DEGs (Ellegren and Parsch 2007; Bonduriansky and Chenoweth 2009; Harrison et al. 2015). Conversely, a monomorphic trait would have similar transcriptional profiles and low numbers of DEGs. As a result, a heteromorphic trait arising from a monomorphic condition (type I) or a cross-sexual transfer (type II) would have high numbers of DEGs and therefore be indistinguishable. Similarly, a monomorphic trait arising from cross-sexual transfer (type II) or one that never had an ancestrally heteromorphic form would have fewer DEGs and also be indistinguishable. More advanced computational techniques could move beyond looking for DEGs for comparisons in and across species and sexes, such as gene

network analysis (Delgado and Gómez-Vela 2019). Regardless of the investigative technique, the necessary component is to identify trait-associated genes and how those do and do not change as the trait emerges or undergoes cross-sexual transfer, respectively. Getting clear transcriptomic data will require robust sampling and the application of more advanced statistical approaches. The advantage of transcriptomes is in their comparative context, the ability to track specific gene products and their expression, and linking corresponding HREs to DEG sets.

In cases of putative secondary monomorphism, transcriptional information can help reject the alternative explanation to cross-sexual transfer, convergence. Observed phenotypes may superficially resemble each other yet have different origins. If the transcriptional profile underlying the monomorphic trait is different between the sexes, then it could be surmised that the trait arose independently in both sexes, indicating two distinct type I traits. An intriguing example of this occurs in forest weavers, where male and females sing duets during courtship, yet the implicated brain regions are different sizes and gene expression patterns within those regions are also different (Gahr et al. 2008). Such an outcome points to an independent origin of each sex’s contribution to the song (type I) rather than a co-option of one sex’s pattern by the other (type II). Another example occurs in poison dart frogs; the tadpole transport behavior is ancestrally male biased (Grant et al. 2006), yet in three species within the clade neuronal activity pattern during brood care is shared regardless of the brooding sex (Fischer et al. 2019). Even in the uniparental species, it is possible for the other sex to engage in brood care in certain social situations (Fischer and O’Connell 2020), suggesting that brood care may repeatedly have type II cross-sexual transfer across species, within a species, and even within individuals.

In some cases, a portion of one sex will display phenotypic values typical in the other, which could be cross-sexual transfer (West-Eberhard 2003) and should have similar transcriptional patterns. Ocellated wrasses have three alternative mating tactics, and the males designated as satellite (those that look phenotypically similar to females) have brain expression profiles that are more similar to females than to territorial male phenotypes (Stiver et al. 2015). Two species of *Julidochromis* cichlids with differing pair dynamics (male-larger or female-larger pairings) have a greater number of overlapping genes based on dominance than based on sex (Schumer et al. 2011). In *Astatotilapia burtoni* cichlids, females can engage in social dominance structures like their male counterparts, where dominant females exhibit similar gene expression patterns in the brain compared with dominant males (Renn et al. 2016). These all could be the result of cross-sexual transfer, but in all cases the possibility exists that the phenotype shared between

sexes was ancestrally shared. Without information on the signal, the HREs, or the ancestral condition, the transcriptional information is insufficient to suggest whether cross-sexual transfer occurred, but there would be a set of genes that putatively underlie the targeted trait that could be used as end points in the regulatory process.

Hormone Response Elements

The AMH predicts that a type I heteromorphic trait should have an increase in HREs associated with trait-associated genes compared with the monomorphic ancestor and that following cross-sexual transfer these HREs are conserved between type I and type II. The initial expectation is the increased HRE motifs will be those associated with the gonadal hormones circulating at higher levels in type I and those HREs will persist in type II. Even though HREs are the end point of the signal, the AMH posits that these are inflexible to change in comparison to the signals (West-Eberhard 2003). Unlike signals, HREs are static over the individual's lifetime and therefore unresponsive to environmental factors. Furthermore, small changes to the signal pathway over time are more likely than wholesale changes from one form to another in the large number of HREs built up from sexual antagonism across the genome.

The bridge from the signal to the transcriptomic suite shaping the observed phenotype are the *cis*-regulatory elements. These are the points where the signal—in this case, a nuclear hormone receptor—physically interacts with the genome to promote or inhibit expression. Evidence is building that these regulatory elements could be major drivers of phenotypic change (Wray 2007; Wittkopp and Kalay 2012). In flies, variation in these regulatory elements can shape the variation in coloration in females (Bastide et al. 2013), as changes to these short sequences can alter specificity and affinity to associated nuclear receptors (Hahn 2007). There are a wide range of regulatory elements present in the genome, but since the focus of the AMH is on the sex-biased signals, the specific regulatory elements to measure are the HREs associated with androgens (AREs) and estrogens (EREs).

As a heteromorphic trait becomes more fixed over evolutionary time, the number of HREs associated with those underlying genes may increase and therefore entrain the regulatory pathways of the trait to the governing sex-biased signal. Heteromorphic traits under the influence of gonadal steroids can have more HREs near the genes associated with the trait that have sex-biased expression compared with a random set of genes (Anderson et al. 2020; Anderson and Jones 2022). This abundance can be caused by duplications of HREs near target genes (Geserick et al. 2005) or novel HREs creating new opportunities for regulation (Jiménez-Delgado et al. 2009). In primates and mice, the gain and loss of proximal HREs can affect sex-biased expression

(Matthews et al. 2021; Anderson and Jones 2022). An intriguing finding is that sex-biased expressions of gene sets that are conserved across populations also have conservation of HREs associated with those genes (Frankl-Vilches et al. 2015; Matthews et al. 2021), yet novel sex-biased expression of gene sets have comparatively fewer associated HREs. All of these findings suggest that while evidence does point to an increase in HREs associated with gonadal steroids (Anderson and Jones 2019, 2022; Anderson et al. 2020), an increase in multiple types of HREs associated with gonadal steroids can occur (Frankl-Vilches et al. 2015; Matthews et al. 2021), as signals can both enhance and repress expression as a means to generate sex-biased gene expression.

With a gene set in hand from transcriptomic work, it is possible to investigate the influence of HREs on the gene expression. Computational techniques exist to scan for HREs in the genome, if one is available, that rely on various assumptions about the affinity of a motif for a given nuclear receptor (Tan and Lenhard 2016; Wang et al. 2016; Anderson and Jones 2019). A more direct technique is chromatin immunoprecipitation sequencing (ChIP-seq; Johnson et al. 2007), which can detect active binding by a nuclear receptor instead of putative locations where binding might be possible as determined by the motif scanning methods.

Signal

Circulating Hormones

Gonadal hormones can resolve genomic sexual antagonism (Ketterson et al. 2005; Cox et al. 2017; Wittman et al. 2021); thus, many novel sexually heteromorphic features would be expected to fall under the predominant gonadal hormone of the sex with high trait values (type I). There is a long history of research into the effect of heteromorphic gonadal hormones on sexually heteromorphic traits, so much so that there is no shortage of examples for this relationship, as listed previously. A one-to-one relationship of circulating levels of the main gonadal hormone to trait expression occurs frequently (Degani 1993; Hews et al. 1994; O'Connor et al. 2011; Rose et al. 2022b). In some cases researchers are able to link the genes responsible for trait formation to the circulating hormone (Khalil et al. 2020), or a comparative context can show that an increase in circulating hormones can lead to differences between closely related species (Hews et al. 2012). These are the cleanest examples for demonstrating a type I trait. Care should be taken to not presume the ancestral condition of which sex has high trait values for the novel trait or how hormonal regulation should proceed. Hormones can function as suppressors instead of enhancers as both directions can result in sexual heteromorphism, which we discuss later.

The more intriguing aspect of hormone levels is how they are involved in cross-sexual transfers resulting in type II traits. Two reviews have discussed how circulating hormones can shape traits in sex-role-reversed species (Eens and Pinxten 2000; Lipshutz and Rosvall 2020a), and they point out that there are few, if any, solid examples of species with higher levels of androgens in females than males or estrogens in males than females. Nonetheless, type II traits are influenced by naturally circulating levels of the gonadal hormone found at lower levels. Aggression, via the challenge hypothesis (Wingfield et al. 1990), is one trait that putatively shows type II in females, with some examples including lizards (Cooper and Crews 1987) and fish (Munro and Pitcher 1985; Desjardins et al. 2006), with more possibilities discussed by Rosvall and colleagues (Rosvall et al. 2019). Aggression also can show type I patterns in females as female bluethroats engage in singing responses to female intruders, which correlates with circulating estrogen levels (Pärn et al. 2008). Goymann et al. (2019) suggest that the challenge hypothesis might better reflect interactions between males and females, which could explain why both males and females in sex-role-reversed spotted sandpipers have elevated testosterone during courtship (Fivizzani and Oring 1986) yet testosterone does not seem correlated with social status or aggression in other reversed species (Koren et al. 2006; Goymann et al. 2008; Muck and Goymann 2019; Lipshutz and Rosvall 2020b). Other examples of traits apparently regulated by gonadal hormones found at lower levels for that sex include the throat patch in female barred buttonquails (testosterone; Muck and Goymann 2011), male satellite morph in bluegills (estrogen; Knapp and Neff 2007), wingspur length in female jacanas (testosterone; Lipshutz and Rosvall 2020b), nuptial coloration in female phalaropes (testosterone; Johns 1964), and arm waving and head bobbing by females to avoid male conflict in Lake Eyre dragons (testosterone; Jessop et al. 2009). In these cases, circulating levels do not have to reach levels found in the other sex, which suggests that a slight increase in levels compared with the ancestral species is sufficient to reach a “threshold” for trait expression (Staub and De Beer 1997). A clear example of this is in white-shouldered fairywrens, where some populations have females that bear the white shoulder ornament typical of males. Females from these populations have higher circulating testosterone compared with females in populations that do not bear this ornament (Enbody et al. 2018).

One consideration should be taken when observing circulating hormone levels; hormones, both endogenous and those from nongenetic parental effects, can influence trait formation later in life (Baroiller et al. 1999; Ketterson et al. 2005; Groothuis and Schwabl 2008; Guiguen et al. 2010; Lipshutz and Rosvall 2020a; van der Bijl and Mank 2021), which can account for incomplete effects of exogenous

hormones on trait formation and transcriptomic profiles (Partridge et al. 2010; Lindsay et al. 2016; Anderson et al. 2020); thus, the “when” (i.e., organizational vs. activational effects) is as important as the “what” when measuring sexually heteromorphic traits. This could mean that at some developmental point, relative circulating gonadal hormones could possibly flip. One intriguing example is in captive hyenas, where neonate females have higher circulating androstenedione, a weak androgen that masculinizes genital formation in rats, compared with males, which may putatively contribute to the formation of the female pseudopenis and later aggression (Glickman et al. 1987, 1992). This finding has not been recapitulated in wild animals, although neonates were not sampled, and no experiments have confirmed the relationship between androstenedione and the female pseudopenis or aggression (Goymann et al. 2001). This example highlights the challenges of hormone sampling: without manipulations, measurements of multiple possible hormones, or explicit timing, hormone levels are correlative, and more evidence is needed for causative relationships that can be supported with other aspects of the AMH.

Metabolic Proteins

Both type I and type II traits rely on identifying the gonadal steroid signal involved in regulating the phenotype. Steroid hormones have complex metabolic pathways, ushered along by various enzymes altering forms that can be more or less responsive signals and even switch gonadal steroids from androgens to estrogens (Baker 2004a; Jasuja et al. 2005; Mindnich et al. 2005). As a result, while circulating hormones may correlate with or seemingly activate the focal trait, they may not represent the true signal at the cellular level. An example of a potential misleading result is in deer mice, where males engage in the female-biased behaviors of grooming and huddling with pups, which correlate with circulating testosterone (Trainor and Marler 2002). Adding inhibitors to aromatase, which converts androgens to estrogens, stops this behavior, suggesting that estrogen is actually the regulator of this behavior. In this case, male pup grooming is seemingly controlled by the androgen pathway and would be considered a type I trait; in actuality, male pup grooming is regulated by the estrogen pathway, which males access by altering their signal, making it a type II trait. Given that estrogen is known to affect parental behavior in rodents (Siegel and Rosenblatt 1975) and females are the main caregivers, this result fits with cross-sexual transfer. In a more surprising result, pied flycatcher males have agonistic behavior correlated with aromatase rather than circulating testosterone (Silverin et al. 2004). As before, this points to male aggression as a type II trait and female aggression is type I, as it is estrogen mediated.

As noted previously, bluethroat females have estrogen-mediated aggression (Pärn et al. 2008) and bluethroats and pied flycatchers are in the same subfamily; perhaps in this group, the agonistic behavior is ancestrally estrogen mediated.

Aromatase is a common enzyme to focus on, as it converts androgens to estrogens and is thought of as a mechanism to increase trait values in males for traits at high values in females (Schlinger et al. 1989, 1999; Haase and Schmedemann 1992), but how can the reverse process occur with no enzymatic process to convert estrogens to androgens? Enzymes can alter the potency of various forms of androgens, making for a stronger signal in cells that might need greater male differentiation in type I traits, such as male song in canaries (Frankl-Vilches et al. 2015) or male pregnancy in pipefish (Beal et al. 2018). Conversely, these enzymes could optimize the low circulating testosterone in females into a more potent one. In Wilson's phalaropes, females have bright nuptial feathers, but these feathers are responsive to testosterone (Johns 1964). In the skin, where the heteromorphic feathers are generated, females have higher amounts of 5α - and 5β -reductase, which converts testosterone to DHT, a more potent androgen (Schlinger et al. 1989). Such an outcome points to type II control of feathers for females. By examining the enzymes along the steroid metabolic pathway in the target tissues, a clearer picture of what signal is actually affecting the expressing cells will point toward which, if any, sex-biased signal truly regulates the trait.

Hormone Receptors

Much like steroid metabolic enzymes, hormone receptors can alter a cell's response to a steroid, and it only requires one or two genes to alter their expression profile. We focus on the two hormone receptors associated with androgen and estrogen signaling: the androgen receptor (AR) and the estrogen receptor (ER), respectively. The receptors can bind many forms of their respective hormones, although some forms have higher affinity than others and can produce stronger effects (Saartok et al. 1984). Increasing the number of hormone receptors can enhance the response of a cell to the circulating or intracellular amounts of steroid hormones and could even offset low circulating levels of a hormone (Maruska and Fernald 2010). In a dwarf hamster, female aggression increases during short days yet estrogen levels diminish, suggesting that estrogen may not drive aggression; however, ER expression is highest during this time in brain regions associated with aggression, pointing back toward estrogen control (Rendon et al. 2017) and aggression being a type I trait in females. Comparative studies of wing snapping in manakins (Pease et al. 2022), foot-flag waving in splash frogs (Mangiamele et al. 2016), drumming in woodpeckers (Schuppe and Fuxjager 2019),

and coloration and aggression in fence lizards (Hews et al. 2012) all demonstrate that as a heteromorphic trait occurs in males, there is a corresponding increase in AR expression. In a similar pattern, rat lines with more female grooming had higher amounts of ER expression in the associated brain regions (Champagne et al. 2003). In birds, brain regions associated with singing can have high amounts of AR and ER expression (Frankl-Vilches et al. 2015), and species with rarely or nonsinging females have greater differences in receptor expression between the sexes (Rose et al. 2022b). All of these examples suggest that receptor expression is a path to heteromorphism and type I patterns by enhancing a cell's responsiveness to sex-biased signals. We suggest that increasing HREs in the genomic architecture would establish more regions to be bound by receptors, which could necessitate an increase in receptor expression to meet the genomic "demand" to generate the sexually heteromorphic trait.

Hormone receptors are also implicated in type II traits. Since reversing the relative level of circulating gonadal hormone levels can cause developmental issues, changing the cellular level, discussed above, or just the cell's responsiveness to available gonadal hormones is a small change that can alter the sensitivity to lower circulating levels of gonadal hormones. In both black coucal (Voigt and Goymann 2007) and barred buttonquail (Voigt 2016) females, two species with sex role reversals, brain regions associated with aggression have higher amounts of AR expression compared with males, suggesting that aggression would be a type II trait in these species. This finding is particularly illuminating in barred buttonquails, where exogenous testosterone in females has no effect on aggression, nor does circulating testosterone in females change following a challenge (Muck and Goymann 2019).

Testing the Ancestral Modulation Hypothesis

The main objective of the AMH is to generate an integrated picture of the regulation of sex-biased traits and use evolutionary history to predict those regulatory pathways. Ideally, for a given trait, one or more species would need to be measured from three groups (fig. 2): a species with ancestral monomorphism, a species with primary sexual heteromorphism, and a species with cross-sexual transfer (either reversed sex expression or secondary monomorphism). Gene expression patterns should show the individuals with larger trait values having more shared expressed genes than in the ancestral species and the individuals with lower trait values. Those shared genes should have an increase in HREs compared with random gene sets in the bearing species (both type I and type II) compared with those same genes in the ancestral species. Several testable predictions emerge from this described pattern.

1. The trait in the initially heteromorphic sex (*a*) should be regulated by the gonadal hormone at high circulating levels for that sex and (*b*) could have an increase in associated receptors or biosynthetic enzymes converting the hormone into a more potent version. There should also be (*c*) a corresponding increase in HREs responsive to the gonadal hormone from 1*a* for novel sex-biased genes compared with the ancestral species.

2. The trait for the cross-sexual transfer sex and species should be regulated by the same hormone as in initially heteromorphic species, but does so by (*a*) an increase in circulating levels of that hormone, (*b*) conversion of circulating hormones into a more potent version or matching type I species through biosynthetic enzymes, (*c*) an increase in receptors associated with type I species, or (*d*) some combination of the previous three situations. There should also be (*e*) no change in the HREs associated with trait expression compared with the type I species.

With all of the possibilities for mechanistic changes in type I and type II traits there could be some underlying pattern when a mechanistic alteration is more likely than others (e.g., increasing nuclear receptor expression), but such a pattern is not readily apparent to us, and we suggest all possibilities be considered while testing.

Finding all three conditions of the trait (ancestral monomorphism, sexual heteromorphism, cross-sexual transfer) for a known evolutionary history is challenging, but having two species for comparisons is a start. Some examples using a comparative and integrated framework include a study for canary song where investigators studied transcripts, HREs, hormone receptors, biosynthetic enzymes, and circulating hormones and did some comparisons to zebra finches (Frankl-Vilches et al. 2015) pointing to what we believe is a type I pattern. Another study examined populations of white-shouldered fairywrens in which females evolved from a nonornamented condition to a male-biased phenotype in some populations (Enbody et al. 2022), which is a cross-sexual transfer. The ornament was testosterone mediated in both males and females with some overlap in genes, although no signal of AR differences pointing toward the expected type II mechanism. Other studies have pointed to species groups where mechanistic patterns are lacking, but there are clear comparative contexts to investigate shifts in sex-biased traits, such as hummingbirds, where females will sometimes adopt the coloration found in males across various species (Diamant et al. 2021; Falk et al. 2021). These groups offer fertile ground to test the evolutionary history and its relationship with repeated trait formation and cross-sexual transfer. Other studies, both those that have been described above and elsewhere in the literature, report just a few of the multiple levels the AMH. These studies have offered a piece of the larger regulatory picture and are opportunities to complete the

puzzle of the mechanistic process from architecture to signal for those traits.

Once more fully validated, the AMH could serve to infer the evolutionary history of a trait based on the regulatory information about that trait formation (e.g., female phalarope color is shaped by androgenic controls), even in the absence of a comparative framework. We caution against hypotheses in this direction until more robust results emerge from multispecies comparative works. An excellent study demonstrating the possibility of single-species studies was done with anoles viewed at all levels of regulation (hormones, receptors, response elements, transcriptome) to determine how androgens shaped phenotypes, which could point to these phenotypes initially emerging in males in a type I pattern (Cox et al. 2022).

Other Considerations

On Mallards and Repression

Hormonal regulation can not only serve to enhance sexually heteromorphic trait expression in the bearing sex but could also serve to repress expression in the nonbearing sex. Like all Galloanserae (land- and waterfowl), feather coloration in mallards is mediated by estrogens (Haase and Schmedemann 1992; Kimball and Ligon 1999) such that male nuptial coloration is repressed by estrogens. During the nonbreeding season when males take on the female-like “eclipse” morph, males convert testosterone to estrogen putatively via aromatase. A study within Galloanserae (Harrison et al. 2015) found that increasing number of DEGs in the spleen and gonads, composed of roughly equal male- and female-biased genes, correlated with increased heteromorphism, but these changes primarily occurred in male transcriptomes. If the same pattern occurs in genes associated with plumage in males, then this suggests that the bright plumage is the novel trait (i.e., type I) but the regulatory pathway is largely through estrogen repression; however, this is only speculation without transcriptomic evidence from the feather follicles themselves. On the whole, it would appear that the male eclipse morph, a form of cross-sexual transfer, is a type II trait and is a form of secondary monomorphism. The interpretation of the AMH would then be that the male coloration is the ancestral state and female coloration is the derived form, which is in contradiction with the fact that the male form drives the DEGs. It could be that selection has acted more strongly on females in the ancestor to escape the developing bright plumage phenotype and once the conflict was resolved via estrogens, genes that underlie the male trait were free to evolve without further need for sex-biased regulation. Such an evolutionary path would still suggest that nuptial plumage in males is a type I trait with its unique transcriptome and evolution of EREs to

repress trait expression in females, while the winter or eclipse plumage is type II, as males access the female morph using existing architecture. Without knowledge of the ancestral morph of all Galloanserae, the AMH cannot satisfactorily explain this pattern. We suggest that examples of repression mechanisms are a challenge to our current narrow definition of the AMH, but further work in these systems could determine whether the AMH as written does explain or might need to be refined to accommodate these systems.

Whiptails and Progesterone

An intriguing case study in the applicability of the AMH can be found in whiptail lizards, particularly the comparison of the ancestral trait-bearing, sexually reproducing *Cnemidophorus inornatus* to the derived, parthenogenic *C. uniparens*. In *C. uniparens* females alternate between receptive behavior and mounting behavior, which correspond to behaviors in *C. inornatus* (Crews and Fitzgerald 1980). Using the AMH framework, the prediction would be that the mounting behavior found in males would have the same genomic patterns and HREs as those found in parthenogenic females, but the signaling pathway would be altered to access the genomic structure. What is seemingly contrary to the AMH is that testosterone modulates male mounting behavior in *C. inornatus* (Lindzey and Crews 1986; Moore and Crews 1986), yet progesterone modulates the mounting behavior of females in *C. uniparens* (Grassman and Crews 1986). The brain regions and biochemical responses associated with the behaviors are shared (Kingston and Crews 1994; O'Connell and Crews 2022), which rules out the initial proposal of convergence in the trait. Androgen levels in *C. uniparens* are undetectable no matter the behavioral stage (Moore et al. 1985) yet exogenous testosterone does initiate mounting behavior in *C. uniparens* (Gustafson and Crews 1981), suggesting that there is an ancestral response to the hormone even if the circulating levels cannot, on their own, generate the behavior. Progesterone can be converted into androgens, and within the brain region most responsible for mounting behavior, the preoptic area of the hypothalamus (POA), *CYP17* is upregulated in *C. uniparens* (Dias et al. 2009), suggesting local enhancement of androgens. Estrogen, androgen, and progesterone receptors are all expressed in the POA in *C. uniparens* (Young et al. 1994; Godwin et al. 2000). Progesterone-induced behavior and gene expression in sexual male *C. inornatus* suggest an ancestral role for progesterone (Lindzey and Crews 1988). Furthermore, both androgen response elements and progesterone response elements exist near a gene promotor associated with mounting behavior (O'Connell and Crews 2022). We suggest that a possible explanation is that parthe-

nogenic females are using an androgenic pathway via cell-level conversion to androgens and that progesterone in both species are converted to androgens to generate the mounting behavior. Another outcome could deal with the nonspecificity of the AREs themselves. AREs are not specific to ARs and can be bound by glucocorticoid, mineralocorticoid, and progesterone receptors depending on minor substitutions to the response element (Nelson et al. 1999). We suggest employing ChIP-seq to determine which receptors are bound during gene expression as the next step to disentangling the hormonal action at play. In both cases, conversion of progesterone to androgens and progesterone receptor binding to AREs, the genomic architecture remains unchanged and the signal is altered to access the established architecture, which is the general prediction of the AMH.

Prolactin, Primary Control, and Toolkits

We present the AMH in narrow terms of androgens and estrogens, but many more signals can have sexual heteromorphism. The primary signal we see for initial expansion of the AMH is prolactin, which is implicated in parental care in multiple vertebrates where the caring sex has increased prolactin levels associated with a variety of care behaviors (Oring et al. 1986; Gratto-Trevor et al. 1990; Kindler et al. 1991; Buntin 1996; Páll et al. 2004; Whittington and Wilson 2013; Fischer et al. 2019; Smiley 2019). Of particular interest is the lability of circulating prolactin levels in contradistinction to the gonadal steroids. Within the AMH framework, we see a resolution that does not require using prolactin itself as the control. We suggest that prolactin is itself part of the heteromorphism for the trait. Convergent evolution often uses similar genes or structures to independently evolve similar phenotypes (e.g., electric organs in fishes [Zakon et al. 2008], venom in platypuses and reptiles [Whittington et al. 2008], or camera eye in octopuses and humans [Ogura et al. 2004]), and prolactin has been recruited numerous times in parental care across vertebrates. We can view prolactin and its downstream effects as a “toolkit” (Newman 2006) that functions in various parental care behaviors with variations each time the toolkit is co-opted to generate the phenotype. Both mammals and teleosts have parental care behaviors that are influenced by prolactin, but there is no evidence to suggest the most recent common ancestor of both groups had parental care, as it has evolved numerous times within teleosts themselves (Kölliker 2012). While prolactin may be a common target to contribute to parental care, the occurrence of sexual heteromorphism still requires a sexually heteromorphic signal to resolve intralocus conflict. Prolactin often requires gonadal steroids to prime organisms to respond to it, and depending on the taxa, estrogens and androgens can be that primary

control (Brown 1985; Schradin and Anzenberger 1999; Smiley 2019). The AMH would designate the priming hormone as the ancestral one (type I), and the priming pathway is the true driver of sex differences. Furthermore, the AMH specifically would state that the primary control (estrogen or androgen) would not flip; thus, a different primary control would represent an independent origin of the behavior even if a similar toolkit (prolactin) was recruited to generate the phenotype. While we present how AMH may influence an oftentimes sexually heteromorphic signal, other signals are worth investigating and testing their influence on sexual heteromorphism as well as their interaction with gonadal steroids.

Shifting HREs

The AMH as we describe it posits that the genomic architecture, particularly the HREs, is conserved between type I and type II species. This is based on two primary assumptions: (1) the genomic architecture for a given trait is complex with numerous HREs distributed throughout the responsive genes rather than one or two loci that control all downstream effects (Geserick et al. 2005; Anderson et al. 2020) and (2) changing from one HRE to another would be an unlikely one-step mutation or have an intermediate step with no regulation as one HRE is lost and another is gained and is therefore less parsimonious. For these reasons, we suggest that HREs would be more likely to remain fixed during cross-sexual transfer than to experience wholesale shifts, although we acknowledge that the latter could occur in rare situations. As a type I trait evolves, HREs are repeatedly recruited; thus, in the stages of early cross-sexual transfer, when fewer HREs control a nascent trait, a shift might be more likely. The unresolved questions include what is the number of HREs needed to generate a sex-biased trait, what is the frequency of shifts to a different ligand, and do those shifts occur by *de novo* recruitment or mutations in existing HREs? In the absence of answers to those questions, we contend that the number HREs used in sex-biased trait expression is too high to allow for frequent shifts from one functional HRE to another across an entire gene set. Nonetheless, by investigating the HREs associated with implicated genes, as described in the AMH, researchers will be able to confirm whether they are conserved following cross-sexual transfer.

Concluding Remarks

The AMH describes the regulatory pathways that underpin sexually heteromorphic traits as a result of the evolutionary history of those traits, whether they emerged from a monomorphic condition or transitioned from a heteromorphic condition. We present two important levels, signal and ar-

chitecture, that contribute to sexually heteromorphic and cross-sexual transfer traits. The AMH predicts that evolution initially acts on the architecture to generate sexually heteromorphism to match the already present sex-biased signal, which we call a type I pattern for a trait. When evolution alters heteromorphic trait values via cross-sexual transfer, either as a secondary monomorphism or as complete reversals, the AMH predicts that the architecture remains unchanged but the signal is altered to access the existing architecture, which we call a type II pattern for a trait. The version of the AMH that we present here focuses on androgens and estrogens in vertebrates, but we acknowledge that more possibilities exist for sexually heteromorphic trait regulation and sexual heteromorphism and that cross-sexual transfers occur in a much wider range of organisms (West-Eberhard 2003).

We propose the AMH as a tool to investigate the mechanisms that shape the evolutionary trajectory of sex-biased expression, particularly in cases of cross-sexual transfer as described by West-Eberhard (2003). The AMH is not merely descriptive; it offers a testable prediction regarding which regulatory mechanisms might underlie the trait given the trait's evolutionary history and vice versa. Our description is a simple verbal model of a complex system in sexually heteromorphic traits given the milieu of hormones present in individuals and the continuum of trait expression across sexes. Nonetheless, we suspect that the main drivers of the heteromorphisms observed in vertebrates are the gonadal steroid hormones, whether their effects are ongoing throughout trait maintenance or development or an early activation point in a regulatory cascade. By putting the AMH forward, we aim to provide a framework of study when considering the regulatory pathways and the evolutionary processes that shaped those pathways.

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Data and Code Availability

No new data or code has been generated for this study.

Statement of Authorship

A.P.A.: original conception, wrote original draft; S.C.P.R.: additional conception, editing and reviewing.

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