Environmental Cues and Mechanisms Underpinning Sex Change in Fish

Laura Casas     Fran Saborido-Rey
Ecology and Marine Resources, Institute of Marine Research (IIM-CSIC), Vigo, Spain

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Abstract
Fishes are the only vertebrates that undergo sex change during their lifetime, but even within this group, a unique reproductive strategy is displayed by only 1.5% of the teleosts. This lability in alternating sexual fate is the result of the simultaneous suppression and activation of opposing male and female networks. Here, we provide a brief review summarizing recent advances in our understanding of the environmental cues that trigger sex change and their perception, integration, and translation into molecular cascades that convert the sex of an individual. We particularly focus on molecular events underpinning the complex behavioral and morphological transformation involved in sex change, dissecting the main molecular players and regulatory networks that shape the transformation of one sex into the opposite. We show that histological changes and molecular pathways governing gonadal reorganization are better described than the neuroendocrine basis of sex change and that, despite important advances, information is lacking for the majority of hermaphrodite species. We highlight significant gaps in our knowledge of how sex change takes place and suggest future research directions.

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Correspondence to:
Laura Casas, lauracasas@iim.csic.es

Teleost fishes exhibit the largest array of reproductive strategies among vertebrates and are the only lineage to display hermaphroditism, defined by the presence of both male and female reproductive function in a single individual [Sadovy De Mitcheson and Liu, 2008]. This unique strategy has fascinated scientists for decades, and significant research efforts have been conducted to decipher the biological, ecological, and evolutionary aspects of this labile adaptation within fish [Warner, 1988a; Shapiro, 1989; Ross, 1990; Avise and Mank, 2009; Avise, 2010; Erisman et al., 2013].

Hermaphroditism can manifest in 2 modes. Simultaneous (synchronous) hermaphrodites possess fully functional male and female gonads concurrently although self-fertilization rarely occurs in fishes [Avise, 2010]. They are far less common than sequential hermaphrodites, which change sex during their lifetime. Change can occur from male to female (protandrous), from female to male (protogynous), or serially (bidirectional sex changers).

A recent review provides a thorough updated list of hermaphroditic fish species [Kuwamura et al., 2020], increasing the number of families with confirmed hermaphroditism by one-third compared to the previous inventory [Sadovy De Mitcheson and Liu, 2008]. A total of 462 species from 41 families and 17 orders display this reproductive strategy, accounting for about 1.5% of teleosts [Nelson et al., 2016; Kuwamura et al., 2020].

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88% correspond to sequential hermaphrodites, and there is a striking dominance of protogyny (305 species) over protandry (54 species) and bidirectional sex changers (66 species). The marine realm is the natural habitat of most sequential hermaphrodites, the majority occurring on coral reefs and only 2% of them inhabiting fresh waters [Pandian, 2010; Kuwamura et al., 2020]. By contrast, freshwater species account for 43% of all known fish species although the constraints preventing the evolution of hermaphroditism in this environment are unknown.

Despite the classical view indicating that hermaphroditism has evolved repeatedly and independently in multiple not-closely related lineages across teleosts [Sadovy De Mitcheson and Liu, 2008; Avice and Mank, 2009; Erisman et al., 2013], this might only be true for protogynous species since protandry and bidirectional sex change seem to be restricted to 4 lineages of Percomorpha [Kuwamura et al., 2020].

This review focuses on recent advances in our understanding of the biological processes underpinning sex change in sequential hermaphrodites, emphasizing the molecular aspects regulating a radical transformation of behavior, morphology, and restructuring of the gonads. We provide an overview of the sequential modifications that occur during sex change, from the integration of the cues triggering sex change at the brain level to the subsequent gonadal reorganization, summarizing the current knowledge. Most research to date has focussed on social species, especially the bluehead wrasse, a key model in sex change, and clownfishes to a less extent, but this review includes all known information on sequential hermaphrodites.

**When, Where to, and Why Change Sex?**

The theory of the size-advantage model (SAM) has been widely used to explain the overall occurrence of sex change and to predict the timing and direction of the switch [Ghiselin, 1969; Warner, 1975, 1988b; Munday et al., 2006; Kazancioğlu and Alonzo, 2010; Erisman et al., 2013]. According to SAM, sex change should be favored when larger body sizes increase the reproductive success of the opposite sex over the initial sex. Thus, sex change is timed to maximize the expected lifetime reproductive output of the individual.

The benefits of the model are tightly linked to the reproductive system and social structure of the species [Munday et al., 2006]. Protogynous species tend to display harem polygyny, conforming well to the SAM predictions. The reproductive value for males increases more with size than for the opposite sex since they monopolize reproductive access to several females [Warner, 1988a; Lutnesky, 1994; Erisman et al., 2009; Hodge et al., 2020]. The largest female of the group undergoes sex change following the disappearance of the socially dominant male. Protandry is favored when reproductive fitness for females increases more with body size than for males. It has been typically reported in monogamous species but also in species displaying random mating with low sperm competition (e.g., several sparids) [Sadovy De Mitcheson and Liu, 2008]. Both are consistent with the SAM expectations although males need a disproportionally high investment in sperm production to compensate for their reduced size and allow fertilization of highly fecund females [Molloy et al., 2007; Pla et al., 2020]. In protandrous and monogamous species, the timing of sex change is controlled socially and follows the loss of the dominant female. In species displaying random mating sex change is often associated with reaching a threshold body size or critical age [Warner, 1988b; Pla et al., 2020].

Although the SAM model explains patterns of sex change in many sequential hermaphrodites successfully, several exceptions do not conform to theoretical SAM expectations. The largest females of some protogynous species do not always change sex [Lutnesky, 1994; Cole and Shapiro, 1995; Munoz, 2004; Clifton and Rogers, 2008; Avice and Mank, 2009], and monogamy has been described in various protogynous species [Kuwamura et al., 2020]. In addition, the patterns of sex change in bidirectional hermaphrodites are not always consistent with SAM. Bidirectional sex change might be favored under high risk of predation that prevents the movement to find new breeding opportunities, resulting in a substantial loss of reproductive output. It also appears to be related to unpredictable changes in social organization [for an extended review, see Munday et al., 2010]. These deviations suggest that sex change in fish might be more complicated than originally anticipated by the model. The incorporation of several factors such as sperm competition have been proposed in order to adjust the model to all the patterns seen in nature [Muñoz and Warner, 2003; Pla et al., 2020].

**Environmental Cues Triggering Sex Change and Their Perception**

Environmental cues triggering sex change differ among species but are typically either size (and/or age) dependent or due to changes in social structure [Godwin, 2009; Kobayashi et al., 2013]. Most protandrous species...
need to attain a critical age or size to initiate sex change, and eventually all individuals in a population will transition to females. Clownfishes (subfamily Amphiprioninae) constitute an exception since sex change is controlled socially. Protogynous species display mostly polygynous mating systems, and sex change is mediated by variations in the social context [Warner, 1984; Kuwamura and Nakashima, 1998; Munday et al., 2006]. The sex ratio of a social group, the demography (size distribution), the density, and the biomass are all factors that affect the dynamics and timing of sex change producing significant variation within and between species [Warner, 1988a; Warner and Swearer, 1991; Buston, 2003; Munday et al., 2006; Ferreira et al., 2010; Taylor, 2014; Chen et al., 2020a]. Detailed studies are lacking for most sequential hermaphrodites, and even less is known about the perception of these environmental cues. In socially controlled sex change, individuals depend on social stimuli, e.g., disappearance of dominant conspecific(s), to trigger the transformation, but the basic functioning of this process remains unanswered. Whether this social stimulus is perceived through the visual, olfactory, auditory, and/or tactile systems is yet a major challenge (Fig. 1). The little information we have derived mostly from studies on social status and courtship behavior in gonochoristic fish, mainly in cichlid species [Escobar-Camacho and Carleton, 2015]. Visual cues alone in Astatotilapia burtoni significantly influence behavior and reproductive physiology [Grosenick et al., 2007; Chen and Fernald, 2011], but they also use olfactory and auditory cues to signal social rank and during courtship [Maruska and Fernald, 2010, 2012; Maruska et al., 2012; Nikonov and Maruska, 2019]. Another cichlid, Oreoichromis mossambicus, communicates social dominance through odorant signals [Barata et al., 2007, 2008; Simões et al., 2015] and relies heavily on acoustic signaling during courtship [Amorim et al., 2003]. Acoustic communication is indeed widespread in fishes and essential for the reproductive and social behavior in many species [reviewed by Amorim et al., 2015], including protandrous clownfishes [Parmentier et al., 2007; Colleye et al., 2009; Colleye and Parmentier, 2012]. Similarly, a large number of species employ tactile stimulation during courtship [reviewed by Kasumyan, 2011]. In protogynous gobies, visual and olfactory cues have been shown to induce sex change although sex- and species-specific differences were observed [Cole and Shapiro, 1992, 1995; Lorenzi et al., 2006]. Visual, olfactory, and tactile cues are suspected to regulate sex change in protogynous black-spot tuskfish [Sato et al., 2018].

Data from gonochoristic and hermaphrodite species indicate that social communication in fishes is often multisensory, but a comprehensive analysis to elucidate the role of all these signals in sex change is lacking. Visual signals likely play a predominant role in many hermaphrodite species, but the relative hierarchy among sensory modalities is dependent on the social organization and the reproductive strategy. Moreover, differences within species might occur, since the habitat and environmental conditions where the specimens live dictate the effectiveness of the transmission via different sensory channels [Maruska et al., 2019].

**Signaling Axis and the Steroidogenic Machinery in Sex Change**

Environmental cues ensue rapid neurochemical changes in the brain to adjust the behavior of social sex changers within minutes to hours when the dominant hierarchy is altered. Subsequently, changes in the brain are transmitted to the gonads ensuing a dramatic reorganization of the gonadal tissue. Gonadal changes usually take considerably longer [Warner and Swearer, 1991; Godwin, 2009; Casas et al., 2016; Dodd et al., 2019; Todd et al., 2019], and their surgical removal does not prevent or discernibly alter behavioral shifts at the brain level [Godwin et al., 1996; Semsar and Godwin, 2003].
The established view proposes the hypothalamic pituitary-gonadal (HPG) axis as the major signaling pathway regulating sex change in hermaphrodite fish. The HPG axis is ultimately responsible for the estrogen-androgen balance that drives gonadal fate [reviewed by Lamm et al., 2015]. The hypothalamus releases gonadotropin releasing hormones (GnRH) in the brain, stimulating the pituitary to synthesize and secrete the 2 gonadotropins (GtHs) – luteinizing hormone and follicle stimulating hormone – into the blood system, both key in the control of reproduction of vertebrates [Clayton et al., 1982]. Subsequently, GtHs regulate the production of sex hormones in the gonad via their receptors, either follicle cells in the ovary or Leydig cells in the testis [Devlin and Nagahama, 2002; Weltzien et al., 2004; Todd et al., 2016].

In teleosts, 17β-estradiol (E2) and 11-ketotestosterone (11-KT) are the main estrogens and androgens, respectively [Frisch, 2004; Guiguen et al., 2010]. During sex change, remarkable shifts in E2 and 11-KT levels occur, with protogynous and protandrous species showing reverse profiles [reviewed by Godwin, 2010]. Protogynous sex change is characterized by elevated levels of E2, followed by a steady increase in 11-KT paralleled by ovarian atresia and proliferation of spermatagonia [Ohta et al., 2008; Muncaster et al., 2013; Todd et al., 2019]. Conversely, protandrous species show a decrease in 11-KT levels preceding E2 increases across sex change [Godwin and Thomas, 1993; Lee et al., 2001]. Bidirectional sex changers show higher concentrations of E2 in females compared to males but no significant differences in 11-KT [Kroon et al., 2003; Lorenzi et al., 2012]. Several manipulative studies have demonstrated the role of the HPG axis in controlling the gonadal fate through the E2-11-KT balance. Administration of GnRH and gonadotropins alters the perception and integration of social stimuli and ensue sex change. Additional studies are needed to clarify the role of these mechanisms in sex change, establish whether they are evolutionarily conserved in protandrous and protogynous species, and elucidate the degree of interplay exhibited between CaRe-sensitive pathways, the HPI, and the HPG axis.

**Neuroendocrine Mechanisms Underlying Sex Change**

The neuroendocrine molecular mechanisms underlying the perception and integration of social stimuli and how they feedback on the brain to promote fine-tuned behavioral shifts and ensue sex change are yet a major challenge. Most information derives from studies of single or few key genes known to be involved in social behavior or sexual development in vertebrates. More recent analysis of global gene expression using transcriptomics has proved inefficient in revealing changes in expression of known key neural regulators, possibly reflecting the subtle or highly localized nature of neural signals [Casas et al., 2016; Todd et al., 2019]. Several neuropeptides known to act as key regulators of the HPG axis and/or modulation of behavior have been proposed to be involved in sex change in fish. The neurotransmitters arginine vasotocin (AVT) and isotocin (IT) are well-studied modulators of social behavior across vertebrates [Black et al., 2004; Godwin and Thompson, 2002; Weltzien et al., 2004; Todd et al., 2016].
Structural changes in AVT/IT neurons or differential expression has been correlated with social dominance hierarchies and aggression in various sequential hermaphrodites, including several tropical wrasses [Godwin and Thompson, 2012; Thomas et al., 2019; Todd et al., 2019], the bluebanded goby [Black et al., 2004], and clownfishes [Iwata et al., 2010; DeAngelis et al., 2020]. Changes in other neuropeptides such as kisspeptins [Shi et al., 2010] and norepinephrine have also been proposed to regulate sex change but their precise roles remain unclear [Godwin, 2010; Larson, 2011; Lamm et al., 2015; Liu et al., 2017].

The gonadotropin-releasing hormone (GnRH) and their receptors are also well-conserved components of the neural molecular machinery. Their expression fluctuates during sex change in protogynous [Kobayashi et al., 2010], protandrous [An et al., 2008, 2010], and bidirectional [Kobayashi et al., 2009] hermaphrodites, but their patterns are inconsistent across species [reviewed in Lamm et al., 2015]. The brain aromatase, on the other

![Fig. 2. Histological sections illustrating sex change.](image)
hand, is a key enzyme that regulates the balance of sex steroids, and its expression has been shown to increase across sex change in protandrous fish [Casas et al., 2016] and decrease sharply at the beginning of sex change in protogynous fish [Todd et al., 2019].

**Gonadal Restructuring during Sex Change**

Changes in the brain are transmitted to the gonads ensuing a dramatic reorganization of the gonadal tissue. Several restructuring patterns can be found among hermaphroditic teleosts, reflecting the diversity of hermaphroditism types and evolutionary origin [see Gemmell et al., 2019 and references therein].

In many species, juveniles develop only ovaries (protogynous) or testes (protandrous) containing gonadotropin-independent gametes [i.e., oogonia and primary growth (PG) oocytes in females and primary spermatogonia in males]. At the onset of sexual maturation, gamete development becomes gonadotropin-dependent, and females start to produce vitellogenic oocytes and then ovules, while males produce spermatocytes and then spermatozoids. Before sex change, no testicular tissues are detectable in the ovary and vice versa [Sadovy and Shapiro, 1987]. At the time fish begin to change sex, they enter the transition phase [Brown-Peterson et al., 2011], which is characterized by the degeneration of the initial gender tissue, concurrently with the ripening of the terminal gender (Fig. 2a). This is a very complex process at a structural, hormonal, and molecular level as it involves atresia of gametes of one sex and proliferation of gonadotropin-dependent growth of gametes of the other sex. It is still unknown from where and what gonadal cell type the new gonadal tissue originates. After sex change is completed, the initial gender tissue is completely resorbed or only small remnants are visible. This pattern has been observed in protandrous species (e.g., *Centropomus undecimalis*) [Taylor et al., 2000], but it is most commonly observed in protogynous fish (e.g., *Labrus bergylta*) [Villegas-Ríos et al., 2013].

In some species, juveniles develop a bisexual gonad or ovotestis, where testicular and ovarian tissues are found well-separated within the same organ and often, but not always, isolated by connective tissue [Sadovy De Mitcheson and Liu, 2008]. Oogonia and PG oocytes in the ovary and spermatogonia and spermatocytes in the testis are visible. In simultaneous hermaphrodites at the onset of sexual maturation both tissues develop into functional ovaries and testis capable of producing gametes during the same spawning season along the entire reproductive lifetime (Fig. 2b), having therefore a permanent ovotestis (e.g., *Serranus scriba*) [Alonso-Fernández et al., 2011]. Similarly, in bidirectional hermaphrodites ovotestis are permanent, but the gonad is unable to produce gametes simultaneously, and sex change means a mere shift in functionality where one tissue is capable to produce gametes and the other remains inactive [Kobayashi et al., 2005]. However, in sequential hermaphrodites with ovotestis (Fig. 2c) the initial gender tissue degenerates concurrently with the proliferation of the final sex tissue during sex change (Fig. 2d). This is most commonly observed in protandrous species (e.g., clownfishes) [Casadevall et al., 2009; Casas et al., 2016] and likely associated with the costs of producing oocytes. Before sex change, the testis is very active producing gametes; however, the ovary, remaining immature, is not completely inactive since oogonia and PG oocytes proliferate as a preparation for the sex change. Hence, there are hormonal and molecular activities in the whole ovotestis for both sexes.

The timing and rate at which one tissue degenerates and the other develops (having or not having ovotestis) differs greatly among species, from days to months [Thomas et al., 2018]. It depends on the reproductive mode as well environmental and social factors, but most commonly, the degeneration is almost completed before the terminal sex becomes sexually mature or competent.

**Molecular Regulation Underpinning Gonadal Sex Change: Do Protogyny and Protandry Share a Mirrored Molecular Mechanism?**

It is widely recognized that the maintenance of the sexual identity of ovary and testis in vertebrates is dependent on male and female pathways acting antagonistically. Thus, gonadal fate is actively regulated not only by the activation of the appropriate sex-specific network but also by the suppression of the sex-opposing network [Capel, 2017].

Until recently, most studies investigated the role in sex change of single or few genes known to be involved in sex determination and differentiation in vertebrates [Miyaki et al., 2012; Thomas et al., 2019; Peng et al., 2020]. The advent of new genomic technologies enabled transcriptomic studies of global gene expression profiles that significantly contributed to our understanding of how sex change is initiated and regulated at the molecular level. Several studies compared male and female expression in hermaphrodite fish species, providing further evidence of
the importance of well-known molecular players in preserving gonadal sex identity [Manousaki et al., 2014; Liu et al., 2015; Tsakogiannis et al., 2018]. Nonetheless, it was the study of transitional stages across sex change, first in clownfish and later in bluehead wrasse, that led to the unveiling of a large number of novel candidate genes potentially implicated in sex change [Casas et al., 2016; Todd et al., 2019].

Within the feminizing pathway, a fundamental gene for the maintenance of the ovarian function is gonadal aromatase (cyp19a1a), an enzyme responsible for catalyzing the conversion of androgens into estrogens [Guiguen et al., 2010]. A strong female-biased expression and a rapid decrease of aromatase expression levels at the beginning of sex change has been described in protogynous species [Liu et al., 2015; Todd et al., 2019; Tsakogiannis et al., 2019]. Conversely, aromatase expression is sharply upregulated at the onset of male-to-female sex change [Wu et al., 2010b; Ravi et al., 2014; Casas et al., 2016] (Fig. 3). The aromatase gene has been proposed to play a central role in protandrous and protogynous sex changers, acting as the potential switch of gonadal sex change by causing the rise or collapse of estrogen production and consequently activating the feminizing and masculinizing pathway, respectively [Casas et al., 2016; Todd et al., 2019]. Administration of aromatase inhibitors blocks/induces sex change in protandrous and protogynous hermaphrodites, respectively [Lee et al., 2002; Horiguchi et al., 2018; Breton et al., 2019].

Transcription factor foxl2 (forkhead transcriptional factor L2) is also vital for the maintenance of the ovarian pathway and the suppression of the male network [Siegfried, 2010; Georges et al., 2014]. It is involved in modulation of aromatase expression and hence estrogen synthesis, suggesting a feedback loop between both [Wang et al., 2007; Yamaguchi et al., 2007; Guiguen et al., 2010]. This positive loop is reflected in a high correlation of the spatial and temporal expression profiles of foxl2 and cyp19a1a in gonochoristic fish during gonadal differentiation [Baron et al., 2004; Nakamoto et al., 2006] and in protandrous clownfish during sex change [Casas et al., 2016]. Expression patterns in the protogynous bluehead wrasse do not seem to be as tightly coupled, showing only partially overlapping profiles. Aromatase expression is rapidly downregulated in transitioning females, while foxl2 levels are maintained across sex change and only arrested in terminal males [Todd et al., 2019] (Fig. 3). Other key players that regulate ovarian steroidogenesis and are activators of the feminizing pathway are the steroidogenic factor sf1, the steroidogenic acute regulatory protein Star, and the enzyme estradiol 17β-dehydrogenase hsd17b, all involved in steroid hormone synthesis [reviewed by Rajakumar and Senthilkumaran, 2020]. These genes show clear sex-specific differences in expression in both protandrous and protogynous hermaphrodites across sex change (e.g., hsd17b; Fig. 3). Moreover, genes involved in the Rspo1/Wnt/β-catenin signaling (e.g., rspo1, wnt4b, fst), known to regulate ovarian differentiation in mammals [Ungewitter and Yao, 2013], have been implicated in sex change in some hermaphrodites [Casas et al., 2016; Tsakogiannis et al., 2018]. In others, however, an unexpected male-biased expression was detected [Liu et al., 2015], highlighting the need to better characterize their functions in teleosts. Todd and collaborators provided an appealing explanation consisting in the duplication and neofunctionalization of genes involved in sexual pathways [Todd et al., 2019].

The male-promoting network is composed, in turn, of various conserved components in sex determination, testicular differentiation, and spermatogenesis in teleosts. Doublesex and mab-3-related transcription factor 1 (dmrt1) plays a pivotal role in the differentiation and maintenance of the gonadal male identity across vertebrates [Matson and Zarkower, 2012]. Its expression has been described as male-restricted or strongly male-biased in at least 20 gonochoristic fish species [see review by Herpin and Schartl, 2011 and references therein]. Mutations of dmrt1 in medaka and zebrafish lead to either male-to-female reversal or dysfunctional males [Masuyama et al., 2012; Lin et al., 2017; Webster et al., 2017]. Studies in diverse animals from mammals to fish have demonstrated an antagonistic relationship between dmrt1 and foxl2 that is essential for the maintenance of gonadal fate [Matson et al., 2011; Li et al., 2013]. Opposite expression profiles characterize dmrt1 during sex change in protogynous and protandrous species, paralleling closely the development or regression of the testes, respectively (Fig. 3) [He et al., 2003; Huang, 2005; Xia et al., 2007; Alam et al., 2008; Casas et al., 2016; Chen et al., 2019; Todd et al., 2019; Zhang et al., 2019].

The anti-Müllerian hormone (amh) gene is another essential member of the male pathway contributing to male sexual differentiation in fish [Pfennig et al., 2015; Adolli et al., 2019b]. Mutations of amh induce male-to-female change in medaka and pufferfish [Morinaga et al., 2007; Kamiya et al., 2012; Nakamura et al., 2012]. Overexpression causes, in turn, female-to-male reversal in orange-spotted grouper [Han et al., 2018]. Moreover, recombinant amh impairs spermatogenesis and arrests proliferation of spermatogonia in gonochoristic and her-
maphrodite fish [Shiraishi et al., 2008; Skaar et al., 2011; Wu et al., 2015]. Remarkable differences in expression between both sexes have been described in both protogynous and protandrous teleosts, and amh expression profiles throughout sex change follow closely those of dmrt1 (Fig. 3) [Wu et al., 2010a, 2017; Casas et al., 2016; Todd et al., 2019; Zhang et al., 2019].
The expression of *amh* is regulated by several members of the *Sox* gene family, an ancient group of transcription factors that encode key mediators of sex determination and differentiation in mammals. The organization and function of this large family is poorly understood in other types of vertebrates, including fish, but some members are well-conserved and widely distributed. The genes *sox9*, *sox8*, and *sox3* are commonly expressed in the gonads of all fish species studied to date, showing a clear sex-biased expression in hermaphrodite species. The expression of *sox9* has been reported as either male-restricted or as strongly male-biased in at least 10 hermaphrodite species, including protogynus and protandrous fish (Fig. 3) [Manousaki et al., 2014; Liu et al., 2015; Tsakogiannis et al., 2018, 2019]. *Sox3* has shown a remarkable female-biased expression in common Pandora, red porgy, and other 5 sparids, but information in other fish families is lacking [Tsakogiannis et al., 2018, 2019]. *Sox8* has been proposed as an important determinant for the maintenance of testis cell identity in protandrous clownfish (Fig. 3), although its expression is female-specific in the protandrous gilthead seabream, suggesting a variable species-specific role [Casas et al., 2016; Tsakogiannis et al., 2019].

Despite strong evidence of the implication of described molecular players in sex change in fish, the precise organization of the gene network directing sex reprogramming has not been elucidated in any hermaphrodite fish species yet. Novel molecular players have been proposed recently [Casas et al., 2016; Todd et al., 2019], but their functions and possible evolutionarily conserved roles remain unexplored.

**The Epigenetic Regulation of Sex Change**

The sexual phenotype is not only the result of genetic pathways but also of environmental influences and epigenetic regulations [Capel, 2017]. These regulations include several types of epigenetic modifications, specifically DNA methylation, histone modifications, and non-coding RNAs, the first 2 being better studied and understood [for a comprehensive review, see Best et al., 2018]. Few studies have assessed epigenetic regulation of sex change in gonochoristic and hermaphrodite species and a systemic analysis is lacking [for an extended review, see Ortega-Recalde et al., 2020]. Most research to date has addressed the analysis of methylation patterns in individual genes, mainly those with a well-known role in sex change in the gonads (see previous section). Expression of *cyp19a1a* was inversely correlated with methylation levels across sex change in protandrous black porgy [Wu et al., 2016], protogynous ricefield eel, and bluehead wrasse [Zhang et al., 2013; Todd et al., 2019], but not in protandrous barramundi [Domingos et al., 2018]. Similarly, progressive methylation/demethylation of *dmrt1* is observed across sex change in protandrous and protogynous hermaphrodites, respectively [Domingos et al., 2018; Todd et al., 2019].

The most complete study to date used whole-genome analysis in bluehead wrasse across sex change revealing distinctive spatiotemporal epigenetic patterns associated with the transformation of ovaries into testes [Todd et al., 2019]. Key epigenetic modifiers, including members of the DNA methylation machinery (such as *tet1*, *tet2*, *tet3* and *dnmt1*, *dnmt3* genes), were shown to fluctuate throughout gonadal reprogramming. Moreover, other epigenetic mechanisms, including histone acetyltransferases and deacetylases and polycomb group (PcG) genes, were dynamically regulated during female-to-male sex change. These results suggest a genome-wide DNA methylation reprogramming event. Histone modifications have also been linked to initiating and maintaining the gonadal identity in other sex-changing species [Zhang et al., 2013; Tsakogiannis et al., 2019].

Altogether, existing data seem to indicate a key role of epigenetic mechanisms in both maintenance of sexual identity and in reprogramming of sexual fate. However, information is lacking for the majority of hermaphrodite species. Moreover, we have a poor understanding of the mechanistic and synergistic action of these modifications in the gonad, while at the brain level no studies have been undertaken in hermaphrodites. Data from mammals provide evidence of an important role of DNA methylation in sexual differentiation of the brain [Forger, 2016; Ratnu et al., 2017], while recent studies in several gonochoristic fish species suggest an involvement of epigenetic mechanisms in response to social interaction [Best et al., 2018]. In cichlids, socially dominant behavior was inhibited by administration of a pharmacological treatment that reduced global methylation [Lenkov et al., 2015]. In addition, epigenetic reprogramming was identified as a key mechanism underlying differential social behavior in adults exposed to different early life social environments [Taborsky et al., 2013].

**Conclusions**

Despite intense research, we are still in the infancy of understanding the process of sex change from a global perspective. Significant gaps remain in our knowledge of
the perception of environmental cues and how they are mediated by the social context, the mechanisms underlying their integration and processing at the brain level, and the exact roles of well-known players at the gonadal level. Most of the data to date is restricted to the detection of changes in gene expression and needs to be validated by functional experiments. Genome editing technologies offer the possibility of directly targeting and modifying genes to elucidate their contribution to the sex change process. Current knowledge is based on the study of few species, emphasizing the need to extend the scope to other species, emphasizing the need to extend the scope to other sex changers to elucidate evolutionarily conserved networks across hermaphrodites. A deeper insight into the molecular and epigenetic mechanisms governing sex change is essential to understand how this biological process might be affected in a changing environment.

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**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.
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