

Evolutionary Diversity and Turn-Over of Sex Determination in Teleost Fishes

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Environmental sex determination · Hermaphroditism · Sex chromosomes

Abstract

Sex determination, due to the obvious association with reproduction and Darwinian fitness, has been traditionally assumed to be a relatively conserved trait. However, research on teleost fishes has shown that this need not be the case, as these animals display a remarkable diversity in the ways that they determine sex. These different mechanisms, which include constitutive genetic mechanisms on sex chromosomes, polygenic constitutive mechanisms, environmental influences, hermaphroditism, and unisexuality have each originated numerous independent times in the teleosts. The evolutionary lability of sex determination, and the corresponding rapid rate of turn-over among different modes, makes the teleost clade an excellent model with which to test theories regarding the evolution of sex determining adaptations. Much of the plasticity in sex determination likely results from the dynamic teleost genome, and recent advances in fish genetics and genomics have revealed the role of gene and genome duplication in fostering emergence and turn-over of sex determining mechanisms.

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Sexual reproduction in its most basic form does not require distinct sexes, but rather simply the union of 2 gametes of any form. However, sexual reproduction often leads to anisogamy, or size-differentiated male and female gametes, and while anisogamy in theory involves strictly the gametes and the gonads that produce them, the evolutionary process that begins with gametic dimorphism has extended consequences in many animals. Anisogamy triggers an evolutionary cascade that extends beyond the gonad to the soma, as circulating sex hormones involved in gonadal and gametic differentiation also influence somatic tissues and behaviors [Zauner et al., 2003; Ketterson et al., 2005; Mank, 2007; McGlothlin and Ketterson, 2008]. Therefore, whether an individual animal develops as a male or female greatly influences its life experience, as sex can shape the way an individual lives, how it acts [Nottebohm and Arnold, 1976; Datta et al., 2008], and ultimately how it dies [Vandenbroeck et al., 2003; Ivakine et al., 2005; Naugler et al., 2007]. Males and females of the same species may also experience different evolutionary pressures [Mank, 2009] and often correspondingly display vastly different phenotypes [Andersson, 1994]. Sex is therefore an important biological and evolutionary component, and so it is hardly surprising, given the importance of sexual reproduction in a Darwinian fitness context, that elements of sex determining pathways are conserved across the animal kingdom [Volff et al., 2003; Ferguson-Smith, 2007]. That said,

the actual mechanism by which this pathway is triggered varies widely and can be a function of inheritance, environment, or both.

In scientific thinking and research, there is a bias towards sex chromosomes, which is primarily due to the fact that most of the major animal models, including humans, possess them. Sex chromosomes arose once in the ancestor of the therian mammals, and all modern therians possess orthologs from this event [Lahn and Page, 1999; Waters et al., 2005; Potrzebowski et al., 2008]. Sex chromosomes arose independently in the avian ancestor, and all modern bird sex chromosomes are descended from this ancestral event [Fridolfsson et al., 1998; Mank and Ellegren, 2007]. Some of the most popular model invertebrates, namely *Caenorhabditis* and *Drosophila*, also possess sex chromosomes [*C. elegans* Genome Sequencing Consortium, 1998; *Drosophila* 12 Genomes Consortium, 2007]. All this produces a misconception that sex chromosomes are the predominant mechanism by which sex is conferred in animals.

In fact, sex can be determined in a variety of ways, and perhaps no group of animals illustrates this more clearly than the teleost fishes. Since its Triassic origin, this clade has evolved and re-evolved a remarkable array of sex determining mechanisms, including constitutive genes linked to sex chromosomes, multi-locus autosomal triggers, environmental factors including temperature, food, and nest availability, as well as demographic and social influences [Devlin and Nagahama, 2002; Mank et al., 2006]. Furthermore, sex need not be a life-long condition in fish, as many groups are simultaneously or sequentially hermaphroditic [see Avise and Mank, this issue]. Several clades have even abandoned sex entirely and evolved unisexual reproductive modes [Avise, 2008]. Clearly for this group of animals, sex chromosomes are just one of many different options for determining who will be female and who will be male.

This wealth of diversity brings with it several questions that we address in this review. Here we summarize what is known about sex determining mechanisms in fish and then attempt to reconcile this with current genomic and evolutionary theory.

Examples will be given for different kinds of sex determination in teleosts, but for an exhaustive catalogue of sex determining mechanisms, we refer the reader to the work of Devlin and Nagahama [2002]. Similarly, we have previously addressed the broad-scale comparative phylogenetics of sex determination in teleosts [Mank et al., 2006] and so refer the reader there for a comparative evolutionary analysis across the clade.

Types of Sex Determination in Fish

Sex is ultimately determined via a biochemical cascade that initiates the formation of either the male or the female gonad. We concern ourselves here with the ultimate factors that initiate that cascade, typically referred to as sex determinants, rather than the proximate genes in the pathway itself. It is useful to delineate these determinants by whether they are internal (inherited and constitutive) or external (environmental or inducible) to the organism.

Sex Chromosomes and Other Inherited Mechanisms

Internal factors determine sex at conception by inheritance. Sex chromosomes are the most commonly identified carriers of constitutive genetic sex determination and derive originally from autosomes that contain a cluster of closely-linked sex determining genes [Ohno, 1967; Charlesworth and Charlesworth, 1978] and which are often called proto-sex chromosomes. There is selection to suppress recombination between the male and female regions of the proto-sex chromosomes, and this yields a sex chromosomal inheritance pattern and neo-sex chromosomes [Charlesworth et al., 2005]. These neo-sex chromosomes often lack morphological differences at this point, and are homomorphic, or visually similar, in chromosome stains and squashes, which are generally the most common genomic scans. Subsequent extensions of the region of recombination suppression, accompanied by chromosomal inversions on one sex chromosome, will lead to degradation of the sex-limited chromosome [Charlesworth, 1991; Vicoso and Charlesworth, 2006] and the emergence of heteromorphic, or visually distinct, sex chromosomes [Graves, 1998; Charlesworth, 1996]. Sex chromosomes do not, by themselves, determine sex, but rather are an epiphenomenon resulting from the constitutively expressed sex determining genes that they contain.

Sex chromosomes may take either of 2 inheritance patterns (fig. 1). Male heterogamety (where females are XX and males XY) and female heterogamety (where females are ZW and males ZZ) have both evolved multiple independent times in fish [Devlin and Nagahama, 2002; Woram et al., 2003; Mank et al., 2006]. Sex chromosomes have been observed in about 10% of surveyed species [Devlin and Nagahama, 2002], though this is a gross underestimate as proto- and neo-sex chromosomes lack distinguishable karyotypic differences between the large major (X or Z) and small sex-limited minor (Y or W) sex chromosomes. When nascent sex chromosomes are ac-

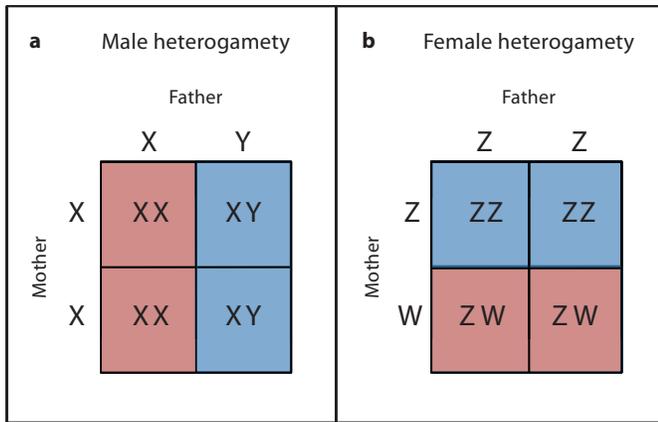


Fig. 1. The inheritance of the minor sex chromosome (Y or W) is correlated with sex. Because the odds of inheriting the minor sex chromosome are even in both cases, the resulting sex ratio is balanced in male heterogametic (**a**) and female heterogametic (**b**) species. In both panels, female offspring are shown in red, male in blue.

counted for, sex chromosomes may be present in up to 50% of teleost species [Arkhipchuk, 1995].

Sex chromosomes alone generally produce a balanced sex ratio, as an individual has an even chance of inheriting the minor sex chromosome from the heterogametic parent (fig. 1). In mating systems with strong monogamous pair bonding, or where female gametes are not the limiting factor in syngamy, a balanced sex ratio is theoretically favored, and sex chromosomes present a stable mechanism to maintain approximately equal numbers of males and females. Additionally, in species where frequency dependent selection acts on sex ratio [Fisher, 1930], such as seen in diadromous fishes that congregate on spawning grounds, a balanced sex ratio conferred by sex chromosomes can provide a stable adaptation. This may explain why sex chromosomes are exhibited in catadromous eels [Park and Kang, 1979; Passakas, 1981] and anadromous salmonids [Woram et al., 2003].

Alternatively, sex chromosome evolution may not be adaptive but rather a product of a more neutral or even relictual genomic process. Evolutionary models without adaptive Darwinian motivation exist, where the emergence of a sex determining gene on an autosome leads eventually to karyotypically distinct sex chromosomes [Charlesworth, 1991; Charlesworth et al., 2005]. This process could produce sex chromosomes even where a balanced sex ratio is not necessarily adaptive. In species where the male reproductive potential outweighs that of females, a balanced sex ratio means that many males may

fail to reproduce. Males will then have to compete for access to females, and this competition may explain the presence of sex chromosomes in lineages with sexually selected male traits and large skews in male mating success, such as the *Xiphophorus* genus [Nanda et al., 2000; Kingston et al., 2003].

Some species have internal constitutive genetic sex determination without sex chromosomes. This can happen when a single gene initiates the pathway, but genetic recombination in the area has not yet been suppressed, and thus the sex chromosomes have not diverged. These are analogous to the fully-fledged sex chromosomes described above, yet are cytologically cryptic. Sex can also be determined by polygenic internal factors, such as the allelic combination of unlinked genes seen in *Dicentrarchus labrax*, the European sea bass [Vandeputte et al., 2007], or the combination of competing female- and male-heterogametic mechanisms that has been shown for some cichlids [Cnaani et al., 2007].

External Influences

Many species of fish delay sex determination until maturation, and this allows a host of external factors to influence sex via inducible sex determining genes. This means that an individual can adopt the sex that confers the greatest expectation of future reproductive success based on the environmental and social factors that are currently available or may be reasonably anticipated, and it is a clever evolutionary strategy to maximize reproductive output in variable ecologies. The external factors that influence sex determination are numerous, and examples are given below.

The temperature of the water column is known to influence sex for a number of species, including the Atlantic silverside, *Menidia menidia* [Conover and Heins, 1987], and likely acts as an environmental gauge. This incorporation of temperature cues into the sex determination pathway could theoretically evolve when the temperature, or the environmental factor that it predicts, affects the reproductive fitness of females and males differently [Charnov and Bull, 1977; Conover and Heins, 1987], as has been empirically demonstrated in reptiles [Warner and Shine, 2008]. A similar situation occurs in some cichlids, except that pH is the environmental stimulus that is correlated with sex [Rubin, 1985]. Presumably, pH in this case is an indicator of some environmental factor with conflicting influence on male and female fecundity.

Demographics can play a strong role in sex determination as well. Particularly for the sequential hermaphroditic lineages, which are described in more detail else-

where [Avisé and Mank, this issue], the sex ratio of the population and the relative position of an individual in the social dominance hierarchy can strongly influence whether an individual behaves and reproduces as a female or a male [Lorenzi et al., 2006; Rodgers et al., 2007]. For hermaphroditic species, demography influences the reproductive fitness of males and females differently, and individuals modulate their sex in ways that potentially enhance personal genetic fitness.

Mixed Strategies

Some species have multiple types of influences on sex determination, combining internal and external cues. For example, several cichlids integrate genetic, environmental, and demographic cues into sex determination [Oldfield, 2005]. Some cyprinids [Fujioka, 2001] as well as the sockeye salmon, *Oncorhynchus nerka* [Craig et al., 1996], have both sex chromosomes and thermal contributors. Such combination strategies may occur as ephemeral transitional states between the different mechanisms, persisting only as long as it takes a newly emergent sex determining mechanism to replace a more ancient form. Alternatively, some mixed strategies may be a form of bet-hedging where variable environments can produce favorable conditions for different types of sex determining mechanisms.

Unisexuality

Several fish lineages have dispensed with sex (and males) altogether, with females reproducing clonally via gynogenesis or hemiclonally via hybridogenesis [Dawley and Bogart, 1989; Avisé et al., 1992; Quattro et al., 1992; Avisé, 2008]. These unisexual biotypes are evolutionarily ephemeral and are invariably confined to the distal twigs of the fish tree of life [Mank et al., 2006]. All known instances of unisexuality in fish originated via hybridization events between related sexual species [Avisé, 2008], and so unisexuality in fish may be the result of non-adaptive processes, such as genomic incompatibilities between the parental species, rather than adaptive evolution.

Presumably, the clonal mode of reproduction for these lineages is a key factor underlying their transience, as unisexual fish lineages are demonstrably less able to adapt to changing ecologies such as emerging pathogens [Hakoyama et al., 2001; Mee and Rowe, 2006] and are more likely to suffer from increased extinction risk due to the accumulation of deleterious mutations and genetic load [Loewe and Lamatsch, 2008]. Sexual lineages produce offspring with a range of fitness coefficients at each round of reproduction, as recombination, random assortment

of genes resulting from meiotic division, and the combination of genes from both parents produces a diversity of genotypes. Emerging ecological challenges screen the suite of offspring genotypes, selecting the most suitable. Clonal lineages must rely on mutation alone to produce variable offspring and cannot assemble different combinations of genes with each generation [Hartl and Clark, 1997], and so are far less able to respond to evolutionary ordeals and may succumb to extinction more easily than their sexual relatives.

The Evolution of Fish Sex Determination

The overall evolutionary picture of sex determination in fish is one of remarkable lability. Even when it appears at first glance that a clade of fishes has a single conserved mechanism of sex determination, further scrutiny often reveals hidden diversity. Evolutionary transitions among all types of sex determination seem possible, although concrete assessments of the rate and direction of transitions have been hampered by the incomplete state of systematic ichthyology and the paucity of information on sex determining mechanism for the clade as a whole. The lability of fish sex determination stands in stark contrast to the strict conservation of single sex chromosome systems in birds and therian mammals, suggesting that some fish-specific character, or suite of characters, must allow fish to transition rapidly to different types of sex determination.

Evolutionary Transitions

Even when a large clade has a single type of sex determining mechanism, further scrutiny sometimes shows that this is due to independent origins of analogous mechanisms rather than a single monophyletic history. For example, whereas most salmonids have XY sex chromosomes, current evidence suggests that they are not all orthologous [Woram et al., 2003]. The same can be said for *Oryzias*, in which multiple sex chromosomes with homologous inheritance patterns have emerged independently [Tanaka et al., 2007]. While the convergent evolution of the same type of sex chromosomes within a clade may seem puzzlingly coincidental at first glance, population genetic models exist to explain the rapid origin of homologous sex chromosomes within a clade [van Doorn and Kirkpatrick, 2007]. This theory suggests that there is a period in the early evolutionary history of a sex chromosome in which linkage between a nascent autosomal sex-determining and sexually antagonistic gene can lead

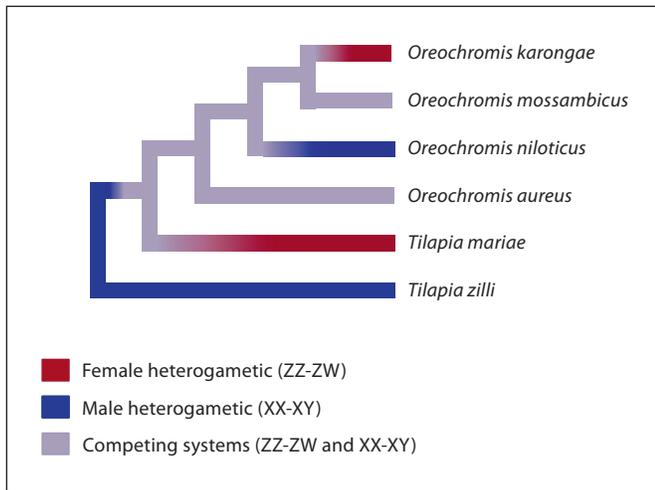


Fig. 2. Rapid transition between sex chromosome types. The tilapia cichlids have transitioned back and forth between female- and male-heterogamety, with an intermediate state containing both types of mechanisms. The reconstruction of sex chromosomal ancestral states is based on maximum parsimony from the available species data, with the assumption that the competing systems of sex determination are orthologous and conserved between *O. mossambicus* and *O. aureus*. Sex determination and phylogenetic data from Cnaani et al. [2007].

directly to the evolutionary emergence of a sex chromosome from that autosome. This process would manifest itself by the presence of clades possessing suites of convergent, similarly aged sex chromosomes.

Possibly the most illustrative example of this in fish is in the stickleback genera *Gasterosteus* and *Pungitius*. In this clade, there has been a recent origin of a male-heterogametic linkage group in the ancestral progenitor, which is present in *Pungitius pungitius*. Following this, there was either a fusion between the *Pungitius* Y chromosome and another linkage group, or a wholly independent turnover on the same linkage group, resulting in an X_1X_2Y system in the branch linking *Pungitius* to *Gasterosteus*. What is most striking about this transition is that both competing male-heterogametic sex chromosomes are present in *G. wheatlandi*, while only the more recent type is present in *G. aculeatus*, making this clade an evolutionary time-series for the origin of, and transition between, convergent sex chromosome types [Peichel et al., 2004, Catherine Peichel, personal communication].

Transitions between female- and male-heterogametic sex chromosome types are also clearly observed from comparative phylogenetic analysis [Mank et al., 2006]. Competing sex chromosomes may exist even within

species, as is the case for the platyfish, *Xiphophorus maculatus* [Volf and Schartl, 2002]. The tilapiine cichlids similarly show rapid transitions between female- and male-heterogametic systems, with several lineages with competing male- and female-heterogametic mechanisms [Cnaani et al., 2007], as shown in figure 2. Evolutionary models have been developed to explain direct transitions between female- and male-heterogamety [Bull and Charnov, 1977; Lande et al., 2001; Vuilleumier et al., 2007]. This theory is based on the invasion of sex reversal genes into a gene pool, which ultimately transform their bearing chromosomes into nascent sex chromosomes of the opposite inheritance pattern. However, these models are predicated on the assumption that such transitions require a concomitant shift from female to male sex determination. In other words, these models assume that females are the default sex in XX-XY species, with a male-determining gene on the Y, and that males are the default sex in ZZ-ZW species, with a female-determining gene on the W. While this assumption seems valid in male-heterogametic systems, where the Y chromosome has been shown to initiate male development in a number of animals [Graves, 1995; Kent et al., 1996], evidence from birds is equivocal as to whether female development in ZZ-ZW systems is linked to the W chromosome [Smith, 2007] or the Z via a sex-regulated locus [Teranishi et al., 2001] or whether the sex of an individual depends on overall chromosome dosage [Ellegren et al., 2007; Itoh et al., 2007].

Evolutionary theory also exists that relates to the factors promoting environmental or conditional strategies. In these cases, the sex determination pathway is theoretically subject to some environmental trigger, such as an inducible promoter. More importantly, this sort of sex determination is adaptive when the environmental or ecological factor has contradictory influences on male versus female fitness [Charnov and Bull, 1977; Conover, 1984]. Conditional strategies can involve body size, as overall mass in many animals, including most fishes, influences the reproductive success of females and males differently, and large individuals will be more successful as one sex compared to the other. This size advantage hypothesis [Ghiselin, 1969, 2006; Warner, 1988], often applied to explain why sequential hermaphroditic fishes switch sex during the course of their lifetime [Lorenzi et al., 2006; Rodgers et al., 2007], also potentially explains how other environmental triggers, such as temperature, can influence sex determination. For example, for species with a sex size advantage, individuals born earlier in the season will be larger than those born later, so when tem-

perature indicates seasonality, its incorporation in sex determination would help maximize an individual's reproductive fitness.

Genomics

The evolutionary theory described above can give clues to how certain methods of sex determination can be favored, but it doesn't answer the questions as to why fish are so labile with regards to this trait. The sex chromosomes in all therian mammals originate from a single evolutionary process [Lahn and Page, 1999; Waters et al., 2005; Potrzebowski et al., 2008] as do the sex chromosomes in all birds [Fridolfsson et al., 1998; Mank and Ellegren, 2007]; yet much younger clades of fish show multiple origins of sex chromosomes [Woram et al., 2003; Cnaani et al., 2007]. What is it about fish that permits such rapid evolutionary change in a trait that it so conserved in other clades?

One distinguishing characteristic is the dynamic teleost genome, which varies greatly in both size and synteny. Gene and genome duplications are an unusually common phenomenon in the teleosts. Indeed, the clade experienced a whole genome duplication at its origin [Hoegg et al., 2004], and there have been subsequent duplications in numerous teleost sub-clades [Mank and Avise, 2006]. These duplications initiate subsequent bursts of reciprocal gene loss, sub-functionalization, and genome reorganization [Brunet et al., 2006; Semon and Wolfe, 2007a, b] that likely form the engine driving many types of teleost diversity, including sex determination [Volff, 2005; Braasch et al., 2007; Siegel et al., 2007].

The dynamic teleost genome likely influences the evolution of sex determination in two different ways. First, duplication of sex determining genes in fish can result in changes in ultimate sex determining mechanisms as the duplicated gene integrates into the sex determination pathway, as has been shown in the medaka, *Oryzias latipes* [Matsuda et al., 2002; Nanda et al., 2002; Kondo et al., 2006]. The duplicated gene can come under a host of new promoters, and this may explain transitions between environmental sex determination and inherited mechanisms. Alternatively, the duplicated sex determining gene can foster the evolution of an entirely new set of sex chromosomes.

The dynamic teleost genome also reshuffles itself far more rapidly than do the genomes of other vertebrate clades. For example, bird genome structure and synteny are highly conserved [Backström et al., 2006, 2008; Stapley et al., 2008], and although mammals do not exhibit the same level of synteny as birds [Thomas et al., 2003],

their genomes are still far more stable than those of the teleosts [Kasahara et al., 2007; Nakatani et al., 2007]. Genome reorganization can influence sex determination by creating nascent linkage groups between sex determining genes and the sexually antagonistic genes that are the basis for much of the theory surrounding sex chromosome evolution [Charlesworth, 1991; van Doorn and Kirkpatrick, 2007]. Such phenomena could explain the repeated origin of convergent sex chromosomes seen in the salmonids [Woram et al., 2003; Phillips et al., 2005] and suggest that a similar process may have occurred in other clades as well.

Implications

With respect to the number and variety of sex determining mechanisms, teleost fishes display a remarkable evolutionary diversity. This is a likely consequence, at least in part, of the dynamic teleost genome. The teleost clade thus provides an excellent model system for testing hypotheses regarding the evolution of sex determination. Many recent advances in fish genetics have expanded our view of what is theoretically and empirically possible, but many questions, such as those surrounding the rate and timing of the transitions among different sex-determining states, remain to be answered definitively.

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