

Review



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# Multiple sex chromosomes in teleost fishes from a cytogenetic perspective: state of the art and future challenges

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Despite decades of cytogenetic and genomic research of dynamic sex chromosome evolution in teleost fishes, multiple sex chromosomes have been largely neglected. In this review, we compiled available data on teleost multiple sex chromosomes, identified major trends in their evolution and suggest further trajectories in their investigation. In a compiled dataset of 440 verified records of fish sex chromosomes, we counted 75 multiple sex chromosome systems with 60 estimated independent origins. We showed that male-heterogametic systems created by Y-autosome fusion predominate and that multiple sex chromosomes are over-represented in the order Perciformes. We documented a striking difference in patterns of differentiation of sex chromosomes between male and female heterogamety and hypothesize that faster W sex chromosome differentiation may constrain sex chromosome turnover in female-heterogametic systems. We also found no significant association between the mechanism of multiple sex chromosome formation and percentage of uni-armed chromosomes in teleost karyotypes. Last but not least, we hypothesized that interaction between fish populations, which differ in their sex chromosomes, can drive the evolution of multiple sex chromosomes in fishes. This underlines the importance of broader inter-population sampling in studies of fish sex chromosomes.

This article is part of the theme issue 'Challenging the paradigm in sex chromosome evolution: empirical and theoretical insights with a focus on vertebrates (Part II)'.

## 1. Introduction

The classical model of sex chromosome evolution posits that they evolved from a pair of autosomes that acquired a sex-determining factor. Sexually antagonistic selection favours the restriction of recombination between the sex-determining and sexually antagonistic loci, which results in the degeneration of sex-specific chromosome Y or W in male- and female-heterogametic systems, respectively. This theory is championed especially by studies of eutherian mammals and *Drosophila* spp. In the latter, the neo-sex chromosomes formed by sex chromosome-autosome fusions of various ages provided an insight into the distinct stages of the differentiation process, which is otherwise difficult to study in evolutionarily old systems [1]. However, the classical paradigm has recently been challenged by examples in other species, most directly in cold-blooded vertebrates [2–5].

Teleost fishes encompass more than half of the extant vertebrate biodiversity [6,7], making this group extremely attractive for studying a variety of evolutionary

subjects, including genome and karyotype evolution. They also represent one of the most diverse animal groups in terms of sex determination and differentiation [8–13]. Fish sex chromosomes typically represent early phases of differentiation with no pronounced changes in their morphology, size and genetic content [8,9,14], although there are exceptions particularly in Neotropical fishes (e.g. [15–20]). This could be owing to the high plasticity of fish sex chromosomes and their frequent turnovers, which repeatedly reset the process of sex chromosome differentiation. This further facilitates the self-enforcing loop between low degeneration and successive turnovers [9,14,21–23].

Nine types of cytogenetically distinct sex chromosome systems with either male or female heterogamety at various stages of their differentiation have been reported in only approximately 5% of the cytogenetically analysed teleosts (based on Arai [24]). However, recent genomic approaches have allowed the identification of small sex-determining regions in many fish species [10,14] using either segregation of single-nucleotide polymorphisms (SNPs) in the F<sub>1</sub> progeny or the association of SNPs with heterozygous sex [25]. Genomic studies point to the presence of homomorphic, i.e. cytologically indistinguishable, sex chromosomes in teleosts and suggest that their incidence is grossly underestimated [14,25–27].

Besides standard constitutions ( $\text{♀XX}/\text{♂XY}$ ;  $\text{♂ZZ}/\text{♀ZW}$ ), fish sex chromosomes include derived systems in which the Y or W chromosome has been lost, i.e.  $\text{♀XX}/\text{♂X0}$ ,  $\text{♂ZZ}/\text{♀Z0}$ , as well as multiple sex chromosome systems  $\text{♀X}_1\text{X}_1\text{X}_2\text{X}_2/\text{♂X}_1\text{X}_2\text{Y}$ ,  $\text{♀XX}/\text{♂XY}_1\text{Y}_2$ ,  $\text{♀X}_1\text{X}_1\text{X}_2\text{X}_2/\text{♂X}_1\text{Y}_1\text{X}_2\text{Y}_2$ ,  $\text{♂ZZ}/\text{♀ZW}_1\text{W}_2$ ,  $\text{♂Z}_1\text{Z}_1\text{Z}_2\text{Z}_2/\text{♀Z}_1\text{W}_1\text{Z}_2\text{W}_2$  (e.g. [28–31]). Multiple sex chromosomes may correspond to: (i) systems with polygenic sex determination, in which alleles from multiple unlinked loci determine sex (based on a cumulative effect) or different sex chromosomes compete in an ephemeral transitional stage during sex chromosome turnover, where one system is epistatically dominant to the other [10,32–34]; (ii) neo-sex chromosome systems which result from rearrangements between ancestral sex chromosomes and autosomes [29,35–38]; and (iii) systems resulting from fissions of the ancestral sex chromosome pair, without the involvement of new autosomal material [39–41].

In the present review, we summarize the current knowledge of multiple sex chromosomes in teleosts and provide new insights into general patterns of their emergence and evolution.

## 2. Multiple sex chromosomes and their importance for research of fish sex chromosomes

In lineages with old and highly degenerate sex chromosomes e.g. mammals and some birds [4,42,43], little can be learned about the factors and mechanisms behind suppressed recombination and sequence divergence between the sex chromosomes, therefore the investigation of younger autosomal additions to sex chromosomes is vital for this type of study [44,45]. In fishes, however, both standard and multiple sex chromosomes often display a low degree of differentiation [8,9]. The analysis of the subtle differences between these sex chromosomes may provide key insights into the evolutionary processes operating at early phases of differentiation [9]. Furthermore, teleosts and ray-finned fishes, in general, encompass various forms of genetically or environmentally driven sex determination (and

the continuum between the two), which can substantially differ between closely related taxa [8–11].

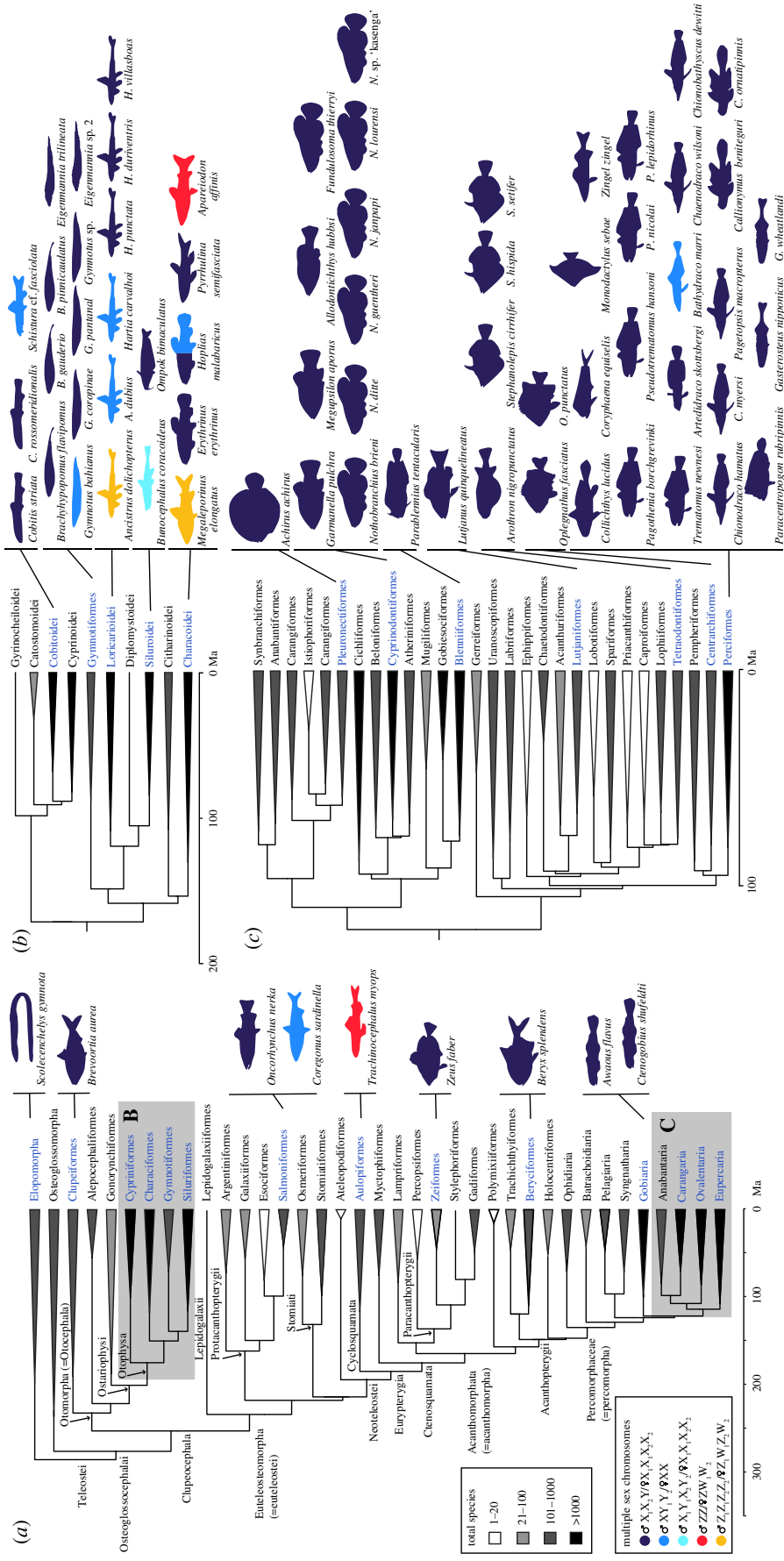
Multiple sex chromosomes can be easily detected by light microscopy as they usually result in different chromosome numbers between sexes [35] and one sex chromosome often notably differs in size and/or morphology. This facilitates more thorough sex chromosome investigations in particular teleost groups, as is the case with sticklebacks (*Gasterosteidae*), which represent the most comprehensively studied teleost taxon concerning the evolution and differentiation of multiple sex chromosomes. This group encompasses three different sex chromosome systems: XY, ZW and X<sub>1</sub>X<sub>2</sub>Y. The latter formed independently in two species *via* sex chromosome-autosome fusions [46,47]. In *Gasterosteus nipponicus*, the fusion brought under sex linkage the genes important for sexual dimorphism and mating behaviour, contributing to its reproductive isolation with its sister species *Gasterosteus aculeatus* [46]. Numerous studies have extended our understanding of sex chromosome differentiation in this model system (for details, see §4a) and showed that multiple sex chromosomes are an extremely interesting and important subject in the systematic investigation of fish sex chromosomes. Yet this has been largely untapped with the exception of two recent reviews compiling information on multiple sex chromosomes in fishes [29,41].

## 3. Multiple sex chromosome constitutions and their distribution in teleosts

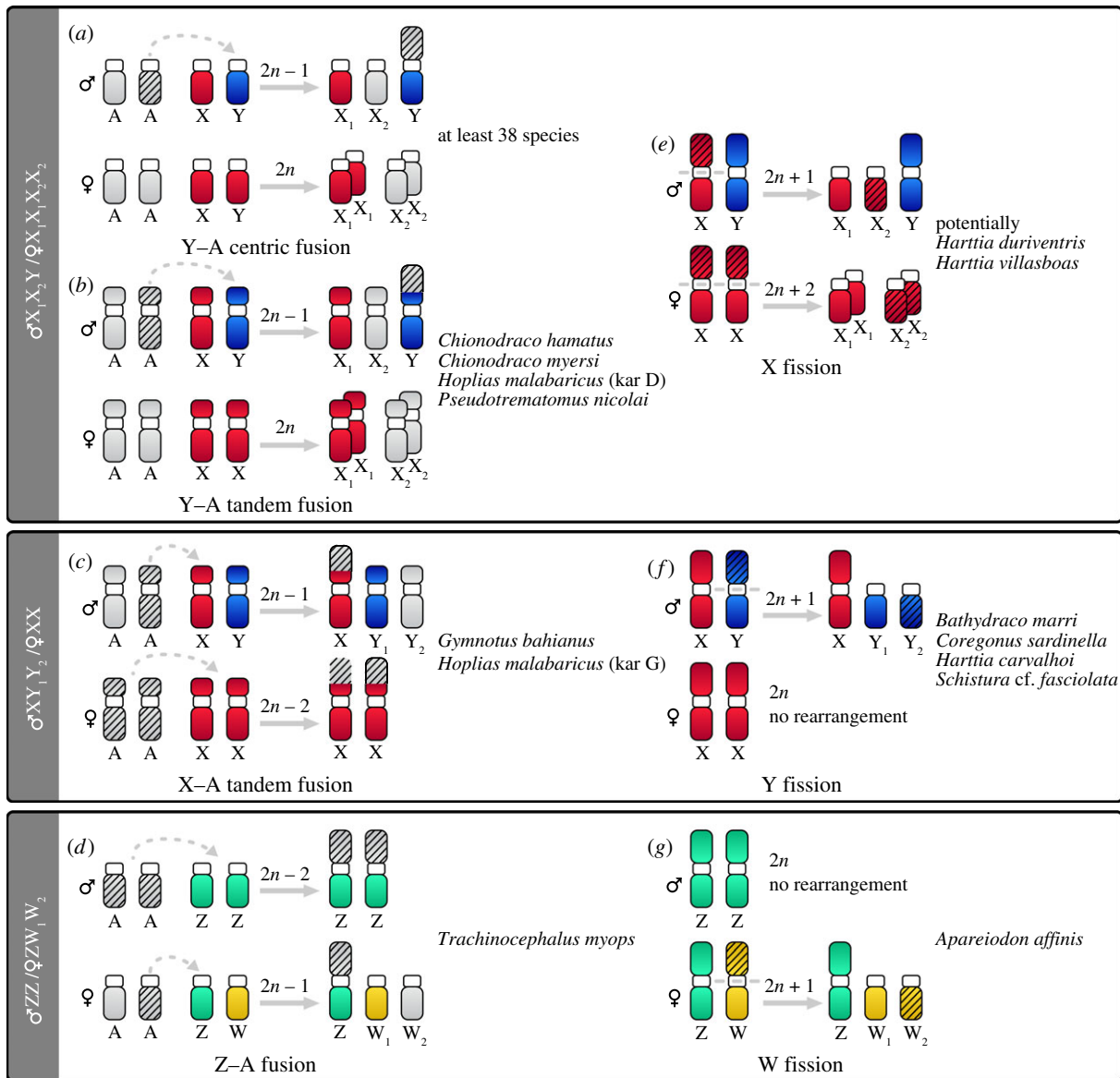
Previously, 47 cases of multiple sex chromosomes in teleosts were recorded, including populations of the same species with different sex chromosome systems [29,41]. The present updated dataset encompasses 75 cases (figure 1; electronic supplementary material, table S1).

The most prevalent multiple sex chromosome system is  $\text{♀X}_1\text{X}_1\text{X}_2\text{X}_2/\text{♂X}_1\text{X}_2\text{Y}$  ( $n = 63$ ; electronic supplementary material, table S1). Among teleosts, it was first described in the monotypic Mexican cyprinodontid pupfish *Megupsilon aporus* [49], a species now considered extinct in the wild [50]. The common feature of this system is the presence of a conspicuously large bi-armed (i.e. metacentric or submetacentric) chromosome exclusive to males. This male-limited sex chromosome is usually formed by a centric or tandem fusion of the ancestral Y with an autosome (Y–A fusion; figure 2a,b), giving rise to a so-called neo-Y chromosome. However, the X<sub>1</sub>X<sub>2</sub>Y system could have also originated from the fission of an ancestral X (figure 2e), although this scenario has been among teleosts only recently proposed in two armoured catfishes of the genus *Harttia* [51]. Another option would be a reciprocal translocation between the  $\text{♂X0}/\text{♀XX}$  system and an autosome pair. However, the resulting neo-Y chromosome would not be conspicuous in size. A comparison of the diploid chromosome number ( $2n$ ) and karyotype structure between closely related species which do not possess multiple sex chromosomes is critical for distinguishing between the above mechanisms (figure 2; [29]), and this also applies to the assessment of the mechanisms discussed below.

Much less frequent ( $n = 7$ ) is the second male-heterogametic system  $\text{♀XX}/\text{♂XY}_1\text{Y}_2$ . It can be formed either by a centric or a tandem fusion between an ancestral X chromosome and an autosomal homologue (X–A fusion; figure 2c), as observed e.g. in one karyotype form of the erythrinid



**Figure 1.** Distribution of multiple sex chromosomes across the teleost phylogeny. (The simplified phylogeny of Teleostei is based on and modified from Betancur-R. et al. [48]. The types of multiple sex chromosomes are distinguished based on the colour of fish silhouettes.)



**Figure 2.** Mechanisms underlying multiple sex chromosome formation in fishes. Seven types of chromosome rearrangements are known to be involved in emergence of the following multiple sex chromosome systems in teleosts:  $\text{♀X}_1\text{X}_2\text{X}_2/\text{♂X}_1\text{X}_2\text{Y}$ ,  $\text{♀XX}/\text{♂XY}_1\text{Y}_2$  and  $\text{♂ZZ}/\text{♀ZW}_1\text{W}_2$ . The mechanisms behind the origin of two remaining systems ( $\text{♀X}_1\text{X}_1\text{X}_2\text{X}_2/\text{♂X}_1\text{Y}_1\text{X}_2\text{Y}_2$  and  $\text{♂Z}_1\text{Z}_1\text{Z}_2\text{Z}_2/\text{♀Z}_1\text{W}_1\text{Z}_2\text{W}_2$ ) need yet to be thoroughly explained. (a) Centric fusion: two uni-armed chromosomes are fused at their centromeric regions to form a larger bi-armed element. (b,c) Tandem fusion: a centromere of one chromosome is fused to a telomere of another chromosome, giving rise to a larger element (herein a bi-armed one). (e–g) Centric fission: characterized by a single break in the centromeric region of a bi-armed element, giving rise to two smaller uni-armed chromosomes. The mechanisms are herein presented in the context of an autosome pair (marked as ‘A’) from which one homologue is fused to a member of standard sex chromosome pair (XY or ZW). The elements undergoing fusion are marked by an arrow (a–c). In the case of centric fission (e–g), the region of breakage on the sex chromosome is marked by a dashed line. Except for the most prevalent system (Y–A centric fusion), examples of teleost taxa are given to each type of mechanism (a detailed list of all teleost taxa possessing multiple sex chromosomes is given in the electronic supplementary material, table S1). The resulting sex-specific change in diploid chromosome number ( $2n$ ) is indicated. In the case of Z–A fusion (d) more specific details about the mechanism are lacking.

wolf fish *Hoplias malabaricus* [52], or by the fission of the original Y chromosome (figure 2f). The latter mechanism seems to be more common (described in four species so far; [53–56]).

A very unusual type of multiple sex chromosome system was documented in the aspeniid banjo catfish *Bunocephalus coracoideus*, where a different karyotype composition between males and females with the same  $2n=42$  suggested the  $\text{♀X}_1\text{X}_1\text{X}_2\text{X}_2/\text{♂X}_1\text{Y}_1\text{X}_2\text{Y}_2$  system [30]. Similar constitutions observed in plants and other vertebrates [37,57,58] were attributed to reciprocal translocations. In *B. coracoideus*, however, crossing between individuals of chromosomal races was proposed. Unfortunately, the analysis of meiotic pairing,

which would test for the expected presence of the sex chromosome quadrivalent, has yet to be carried out. A high rate of chromosome rearrangements was observed on the inter-population level in this species and the  $\text{♀X}_1\text{X}_1\text{X}_2\text{X}_2/\text{♂X}_1\text{Y}_1\text{X}_2\text{Y}_2$  system has not been found in any other populations of this putative species complex [59].

Comparably rare is the female-heterogametic system  $\text{♂Z}_1\text{Z}_1\text{Z}_2\text{Z}_2/\text{♀Z}_1\text{W}_1\text{Z}_2\text{W}_2$  found only in the loricariid catfish *Ancistrus dolichopterus* [28,60] and the anostomid characin *Megaleporinus elongatus* [61,62]. This multiple sex chromosome system has almost no parallel in other organisms with female heterogamy except for butterflies, in which complex derivatives also exist [63].

The second and only other female-heterogametic multiple sex chromosome system reported in fishes is  $\delta ZZ/\varphi ZW_1W_2$ , found in the marine lizardfish *Trachinocephalus myops* [64] and the parodontid characin *Apareiodon affinis* [39,65]. While in *T. myops*, the proposed mechanism of origin is a Z–A fusion [64] (figure 2d), in *A. affinis* it is thought that it emerged from a centric fission of an ancestral W chromosome (figure 2g) followed by additional rearrangements [39,65]. In *T. myops*, the sex chromosomes are highly differentiated as neo-Z is disproportionately large in comparison to its small-to-tiny  $W_1$  and  $W_2$  counterparts [64], or, alternatively, the mechanism of their origin was more complex.

Fish sex chromosomes evolved independently in many taxa, sometimes even within the same genus or species [21,66]. Our compiled dataset mapped together with numbers of taxonomically recognized species (electronic supplementary material, table S2, [7]) on the teleost phylogeny (figure 1; [48]) indicates that multiple sex chromosomes are scattered across the entire teleost clade. From a broader perspective, no multiple sex chromosomes have so far been reported for any of non-teleost ray-finned fishes (Actinopterygii) [13,24], whereas  $X_1X_2Y$  sex chromosomes have been found in cartilaginous fishes (Chondrichthyes) [67,68]. Although sampling bias cannot be ruled out, there are no reports of multiple sex chromosomes in three speciose teleost orders (Stomiiformes, Gadiformes or Ophidiiformes), while these are common in other lineages (electronic supplementary material, table S2). Considering the proportion of species with multiple sex chromosome systems relative to the overall number of species with sex chromosomes in orders where these data are available for more than 10 species (electronic supplementary material, Appendix S1 and table S3), we observed a significantly higher percentage of multiple sex chromosome systems in the order Perciformes (60%; Fisher's exact test  $p = 0.012$ ) compared to the mean (17.9%) across all orders. Within Perciformes, 66.7% of the cases belong to the Antarctic sculpin clade Notothenioidei (namely families Artedidraconidae, Bathydraconidae, Channichthyidae and Nototheniidae; [69,70]). The other orders do not differ significantly from the average. At the genus level, the highest number of multiple sex chromosomes (six) has so far been reported in African annual killifish *Nothobranchius* (figure 1; electronic supplementary material, table S1; [71,72]). Some not well supported or even inconsistent reports, such as in the goby *Awaous flavus* and the doryfish *Zeus faber* (electronic supplementary material, table S1), would benefit from a re-examination, as well as several other speculative cases of putative sex-linked chromosome polymorphisms reported, e.g. in the salmonids *Coregonus albula* [73] and *Salvelinus alpinus* [74].

An important caveat to the present dataset is that more than half of the records are based only on conventional cytogenetic methods, i.e. uniform staining of chromosomes (typically by Giemsa solution), supplemented in some cases by differential staining of constitutive heterochromatin (C-banding) producing characteristic bands (electronic supplementary material, table S1). These methods may determine chromosome counts and morphology; C-banding may additionally reveal differences in heterochromatin distribution between sex chromosomes. However, the accurate identification of all elements of the multiple sex chromosome constitution is often challenging. For example, it is difficult to identify individual uni-armed chromosomes such as subtelocentrics and acrocentrics in karyotypes consisting predominantly of

uni-armed elements gradually decreasing in size, hence,  $X_1$  and  $X_2$  chromosomes in the  $X_1X_2Y$  system are often chosen arbitrarily. Also, without the analysis of meiotic pairing, we cannot reliably differentiate between  $XX/XO$  and  $X_1X_2Y$  systems, which form a univalent and a trivalent, respectively, in the first meiotic division, as male and female  $2n$  differ in the same way in both constitutions. If data on meiotic pairing are not available,  $2n$  and karyotype structure should be compared with several closely related species in order to determine whether chromosome rearrangements such as fusions or fissions have occurred (e.g. [53,75]; figure 2). In addition, the analysis of multiple sex chromosome pairing in meiosis could reveal regions of asynapsis (i.e. mispairing between homologues) as a sign of sex chromosome differentiation [76], which may be further characterized by immunostaining specific proteins of the synaptonemal complex [77]. Numerous studies in teleosts also do not distinguish ancestral sex chromosome elements from the new additions. Chromosome landmarks such as a characteristic block of constitutive heterochromatin [54,78,79] or accumulations of specific repetitive elements traced by fluorescence *in situ* hybridization (FISH) (e.g. [52,55,69,80–82]) are helpful, especially if they highlight a putative region of sex chromosome differentiation. However, as fish multiple sex chromosomes do not display pronounced degeneration, these markers are of limited use and hence a suite of advanced molecular cytogenetic techniques must be used to improve chromosome identification (see §§4a and 4c for details).

Moreover, it has been repeatedly shown that populations of many teleost species and species complexes are polymorphic for sex chromosome systems [31,59,65,70,79,83–89]. These observations point to a very recent, ongoing and recurrent formation of multiple sex chromosomes and stress the importance of proper sampling and sample size. In fishes, initial reports of multiple sex chromosomes have already been refuted using larger sampling [90]. As highlighted in the electronic supplementary material, table S1, around half of the studies had been conducted on a single population, some with only a rather limited sampling.

#### 4. Evolutionary pathways of teleost multiple sex chromosomes: current state of knowledge and further research directions

The number of reports of multiple sex chromosomes in teleosts is ever-growing. Despite our increasing knowledge of mechanisms of their origin in particular lineages, it is yet to be elucidated: (i) what forces drive the evolution of multiple sex chromosomes, (ii) whether it is associated with changes in the sex-determining pathway, and (iii) whether they contribute to species diversification.

##### (a) Genetic content of fish multiple sex chromosomes

Despite some exceptions (see below), it has been repeatedly documented that fish multiple sex chromosomes do not accumulate heterochromatin [17,91,92]. However, how this relates to levels of their differentiation is currently unclear (cf. [93]). Sex chromosome differentiation reflects recombination, yet the empirical data about the recombination landscape of fish multiple sex chromosomes are so far limited solely to sticklebacks [46,47,91,94–96].

Although the absence of substantial blocks of constitutive heterochromatin simplifies sequencing and assembly of unpaired sex chromosomes, male-limited neo-Y chromosomes have only been sequenced in a few species so far. In the spiny-head croaker *Collichthys lucidus*, the recent report identified *dmrt1* as a candidate master sex-determining (MSD) gene [97], while in the barred knifejaw *Oplegnathus fasciatus* [98], a comparison between the male and female assemblies revealed approximately 99% identity between the neo-Y and the X<sub>1</sub> and X<sub>2</sub> sex chromosomes, several rearrangements in the interstitial regions of the neo-Y, and a set of male-specific genes. The extent of neo-Y differentiation has been thoroughly studied in the sticklebacks *G. nipponicus* [95,96] and *Gasterosteus wheatlandi* [94]. In the X<sub>1</sub>X<sub>2</sub>Y system of *G. nipponicus* formed within the last 2 Myr (million years) by a Y–A fusion, recombination between the new sex-linked regions ceased gradually, starting from the fusion point and spreading across a large region of the neo-Y [91]. No clear signs of degeneration of the neo-Y were observed, although its genes started accumulating deleterious mutations [96]. In *G. wheatlandi*, the X<sub>1</sub>X<sub>2</sub>Y system of comparable age evolved independently via fusion of the ancestral Y chromosome with another autosome. The *G. wheatlandi* neo-Y underwent also little degeneration, though its non-recombining region is much larger. Interestingly, the shared ancestral Y chromosome experienced more extensive differentiation in *G. wheatlandi* than in *G. aculeatus* and *G. nipponicus* [94].

As for a putative MSD gene, *G. nipponicus* and *G. wheatlandi* share an ancestral Y sex chromosome with the three-spined stickleback, *G. aculeatus*, in which the *amhy* gene was identified as a potential MSD candidate [94,99]. Similarly, there are strong indications that the neo-Y of sockeye salmon *Oncorhynchus nerka* contains *sdY* as a salmonid-specific MSD gene [100–102]. However, the candidates for MSD function in other teleost fishes with multiple sex chromosomes currently remain unknown.

Despite recent progress in fish genomics, our knowledge of the genetic content of fish multiple sex chromosomes still largely stems only from cytogenetic analyses detecting, to various degrees, differences in molecular composition. Several repetitive DNA sequences were mapped on fish multiple sex chromosomes, which helped to determine their evolutionary origin (e.g. [16,69,82]). Comparative mapping of specific repeats between closely related species can support or disprove the homoeology of sex chromosomes [61,69,80,103] and identify ancestral parts of multiple sex chromosomes [81]. A specific block of accumulated repeats may point to regions of suppressed recombination, breakpoints of chromosome rearrangements, or to remnants of centromeric sequences inside the fused chromosome [61,70,80]. Similarly, interstitial telomeric sequences (ITSs) i.e. telomeric repeats located by FISH inside the chromosome may signal chromosome rearrangements [104] and thus help to trace the mechanism of multiple sex chromosome formation. However, our dataset (electronic supplementary material, table S1) suggests that in eight out of 16 studied cases, ITSs have not been found in the supposed fusion points, which may reflect either their erosion or low copy number falling below the resolution of FISH. In two other species, the ITSs were found on multiple sex chromosomes but were probably not relevant to their origin [75,105]. Nevertheless, FISH with the telomeric probe is also particularly useful for determining a number of chromosomes involved in meiotic multivalents (e.g. [36,106]). More thorough characterization of repeats involved in fish multiple sex chromosome differentiation has been recently enabled by the

implementation of novel bioinformatic pipelines such as RepeatExplorer as exemplified by *M. elongatus* [107].

Comparative genomic hybridization (CGH) might also assess the differentiation of multiple sex chromosomes. Simultaneous hybridization of male and female whole-genome probes to chromosome spreads may reveal differentially painted regions, which can correspond to non-recombining loci accumulating sex-limited or -enriched sequences. To date, this method has been used in diverse fishes with multiple sex chromosomes with varying success [51,52,108–110]. One drawback, however, of CGH is its low resolution as it fails to provide detailed sequence composition of the sex-specific region.

Gene content of fish multiple sex chromosomes can play an important role in their evolution. Sex chromosome–autosome fusions considerably increase the number of sex-linked genes which can contribute to adaptation and reproductive isolation (cf. [111]). Multiple sex chromosomes may thus promote the formation of pre- and post-zygotic barriers in teleosts as seen in sticklebacks [46]. Recently, it was shown that early differentiation of multiple sex chromosomes in *Drosophila miranda* was accompanied by the massive amplification of gene copies leading to their tandemly repeated arrangement on both X and Y chromosomes [112]. Similarly, the amplification of genes linked to complex multiple sex chromosome systems was observed also in wood white butterflies of the genus *Leptidea* [113], suggesting that the mechanism could be a common feature of sex chromosome differentiation. Thus, a combination of gene amplification with the frequent sex chromosome turnover observed in fishes, both possibly induced by increasing mutation load, could systematically create novel selectable variation and thus considerably increase the adaptive potential in fishes (cf. [114]).

### (b) Differentiation of teleost multiple sex chromosomes

There are major differences in the way distinct types of teleost multiple sex chromosomes degenerate. Our dataset (electronic supplementary material, table S1) shows a high prevalence of male heterogamety ( $n = 71$ ) with no substantial degeneration of neo-Y chromosomes detectable by cytogenetic methods. In one specific case, the high heterochromatin content was already present on the ancestral Y chromosome prior to multiple sex chromosome origination [79]. However, while acknowledging the low number of known cases ( $n = 4$ ), W chromosomes in female-heterogametic multiple sex chromosome systems accumulated heterochromatin and/or specific repeats in all cases except for the bushymouth catfish *Ancistrus dolichopterus* [16,28,60,61,64].

The reason for such disparity in the incidence and trajectory of multiple sex chromosome differentiation between male and female heterogamety is not clear. One possibility might be that fish W sex chromosomes degenerate faster in standard systems, restricting the opportunity for sex chromosome turnover. This is corroborated by hybridization patterns of W-specific chromosome probes which usually paint only a small portion of Z owing to rapid W differentiation (e.g. [18,20,62,115]), while probes from male-heterogametic systems usually paint all sex chromosomes equally in the complement (e.g. [52,109,110]).

It must be noted that the majority of cytogenetic reports documenting highly degenerated ZW sex chromosome systems are confined to the neotropical representatives of the order Characiformes, where several monophyletic and

evolutionarily old systems have been described ([15–20]; electronic supplementary material, table S4), allowing the possibility of sampling bias in our general assumption. Moreover, Pennell *et al.* [116] concluded based on formal analysis conducted on data from the Tree of Sex database [13] that there are no differences in the rates of transition from homomorphic to heteromorphic sex chromosomes between XY and ZW systems.

We compiled our own datasets (electronic supplementary material, table S4; for summaries see the electronic supplementary material, Appendix S1—Appendix tables 1 and 2) using various filtering criteria (for details see the electronic supplementary material, Appendix S1) and used them to test the following hypotheses. First, we tested whether XY and ZW systems differ in sex chromosome differentiation. Fisher's exact test identified significant ( $p = 3.7 \times 10^{-7}$ ) excess of homomorphic sex chromosomes in lineages with male heterogamety (65.8%) compared to lineages with female heterogamety (20.9%). We further sought to test our hypothesis that ZW systems differentiate faster in evolutionarily young sex chromosome systems. Fisher's exact test revealed that the proportion of homomorphic cases was significantly higher ( $p = 0.006$ ) in XY systems (88.9%) than in ZW sex chromosomes (33.3%). However, the dataset for the test contained 45 male-heterogametic sex chromosome systems but only six records of female-heterogametic systems. We noted that compared to ZW systems, there are more homomorphic XY systems that escaped cytogenetic detection but were captured by other genetic or genomic approaches (electronic supplementary material, Appendix S1). We decided to compile yet another, hopefully larger, dataset using this as a proxy for level of sex chromosome differentiation. The resulting dataset contained 53 records of XY sex chromosomes with 42 homomorphic systems and nine cases of ZW sex chromosomes with four systems being homomorphic. For this dataset, Fisher's exact test also revealed a significant difference in the proportion of homomorphic sex chromosomes between male and female heterogamety ( $p = 0.0417$ ). It should be noted that the latter dataset could be biased by genomic studies focused mainly on taxa of economic importance. Taken together, we hypothesize that rates of sex chromosome differentiation vary between male and female heterogamety and our results warrant further study.

On the mechanistic basis, one probable explanation for possible faster *W* sex chromosome differentiation may be heterochiasmy, i.e. the difference in rates of recombination and chiasma localization between males and females, which is indeed widespread [95,117–121]. Typically, recombination rates are higher in subterminal chromosome regions in males, while in females recombination is higher in interstitial regions, i.e. across much of the chromosome length [117]. Inversions on sex chromosomes should fix more frequently in ZW taxa under the typical recombination landscapes as selection favours rearrangements proportionally to how much they reduce recombination between sexually antagonistic and sex-determining loci [117]. Another explanation could stem from dosage-dependent male sex determination mechanisms confirmed in some female-heterogametic taxa such as the chicken [122] and the half-smooth tongue sole, *Cynoglossus semilaevis* [123,124]. In these taxa, male sex is determined by the dosage of Z-linked MSD genes. Since there is no MSD gene on a *W* chromosome, it is reasonable to assume that the *W* chromosome is functionally less constrained and under weaker purifying selection, therefore allowing faster

differentiation. Our hypothesis does not exclude the possibility that the *W* chromosome may acquire an MSD gene secondarily, e.g. by transposition. Bearing in mind the difficulties in assembling highly degenerate sex chromosomes [125], transcriptomic studies might be more helpful in testing our hypothesis.

### (c) Possible drivers of the evolution of fish multiple sex chromosomes

The emergence of multiple sex chromosomes represents an interesting diversion from the classical scheme of sex chromosome differentiation as it may substantially change the composition, epigenetic landscape and evolutionary dynamics of these elements. Drivers of sex chromosome turnover, including the formation of multiple sex chromosomes in vertebrates, have recently been comprehensively reviewed [21,29,41,126–128]. Several different scenarios can be applied to teleost species with multiple sex chromosomes.

Our dataset (electronic supplementary material, table S1) suggests that sex chromosome–autosome fusions are by far the most prevalent mechanism giving rise to multiple sex chromosomes in teleosts. Also, the incidence of sex chromosome–autosome fusions is much higher in male-heterogametic than female-heterogametic taxa (Fisher's exact test for all data  $p = 4.4 \times 10^{-10}$ ; non-parametrical ANCOVA adjusting for the total number of sex chromosomes cases  $F_{1,147} = 10.33$ ;  $p = 0.0016$ ; for details see the electronic supplementary material, Appendix S1 and tables S5–S6), thus following empirical evidence from other vertebrates [41,128].

Our compiled data further indicates that Y–A fusions predominate over other types of sex chromosome–autosome fusions (electronic supplementary material, table S7), as previously reported in other cold-blooded vertebrates [41]. The most plausible explanation for the observed pattern would be that sex chromosome–autosome fusions are deleterious and fixed by genetic drift [129]. According to the drift hypothesis, sex chromosome–autosome fusions should be fixed most often in sex chromosomes with the smallest effective population size, i.e. the *Y* and *W* chromosomes. Yet, the dearth of *W*–A fusions in vertebrates is not consistent with the drift ([41]; but cf. [130]). Furthermore, it is important to note that multiple sex chromosomes are not limited only to fishes with small or fragmented populations and low vagility but can be also found in marine lineages with high dispersal potential (e.g. [131]). Pennell *et al.* [41] concluded from their analysis that the prevalence of Y–A fusions in fishes and reptiles can be best explained by a combination of underdominance of the fusions, male-biased mutation rates for fusions and female-biased reproductive sex ratio.

Pokorná *et al.* [128] proposed that female meiotic drive could constrain the incidence of multiple sex chromosomes in female-heterogametic systems. Meiotic drive is the non-random segregation of chromosomes owing to the asymmetry of female meiosis, which results in only one functional gamete, oocyte and three polar bodies [132]. This mechanism affects all chromosomes in the complement and may also contribute to preferential fixation of certain multiple sex chromosome systems. In mammals, for instance, the  $XY_1Y_2$  sex chromosome system created by X–A fusion was shown to be more prevalent in complements dominated by bi-armed chromosomes as the drive favours the bi-armed chromosome morphology. On the other hand, complements dominated by uni-armed elements displayed a higher incidence of the  $X_1X_2Y$  sex chromosome system originated from Y–A fusion

since the Y chromosome never enters female meiosis and therefore is not affected by the drive [133]. When applied to ZW-derived multiple sex chromosomes, meiotic drive would cause a substantial sex ratio distortion in females and is therefore selected against. In our dataset with teleost species, abundance of multiple sex chromosome systems originated by rearrangements of Y chromosomes (50 out of 54 of independent origins; electronic supplementary material, Appendix S1–Appendix figure 1 and table S7), which is never involved in female meiosis, and scarcity of X–A ( $n=2$ ) and Z–A ( $n=1$ ) fusions makes any formal analysis of the effect of meiotic drive on fish multiple sex chromosome formation impossible. We therefore performed a Wilcoxon rank-sum test to assess the association of the percentage of uni-armed/bi-armed chromosomes on the emergence of sex chromosome–autosome fusions and fissions (electronic supplementary material, Appendix S1, Appendix figure 1 and table S7). The association between the type of rearrangement and the karyotype structure was not significant for either males (complete dataset;  $Z=174$ ,  $p=0.128$ ) or females (complete dataset;  $Z=178.5$ ,  $p=0.096$ ). We can conclude that the correlation between a high percentage of uni-armed chromosomes in the karyotype and fusion leading to multiple sex chromosomes is not significant in the analysed teleosts, and inversely, the proportion of bi-armed chromosomes does not correlate with fissions.

It was noted that certain chromosomes are ‘better at sex’ than others [134]. To assess whether sex chromosomes arose independently or by co-option of the same synteny block, FISH with bacterial artificial chromosomes [46,101,135], and whole-chromosome painting with probes derived from specific sex chromosomes [16,62,110,136,137] can be used. For instance, a common origin of multiple sex chromosomes was revealed in two *Oplegnathus* knifejaw species [110] and partial homoeology between different multiple sex chromosome systems was shown in sticklebacks [46,47] and tentatively also in the glass knifefishes of the genus *Eigenmannia* [138].

It has been hypothesized that fusions between sex chromosomes and an autosome enriched in sexually antagonistic genes are favoured by selection [44,139]. Simulations further suggested that sexually antagonistic selection may contribute to the elevated fixation of Y–A fusions when it evolves asymmetrically, i.e. alleles advantageous for males and detrimental for females will be maintained in the population with higher frequency than those with the opposite effect [140]. However, the results gathered so far, especially in ranid frogs, provided no support for the theoretical role of sexually antagonistic genes in the evolutionary dynamics of sex chromosomes [141,142]. Alternatively, different genes involved in the sex-determining pathway can take over the role of the MSD gene and give rise to new sex chromosomes [47,143,144]. Indeed, a handful of genes are being repeatedly co-opted as MSD in fishes [10,123,143,144] and thus may contribute to the repeated use of the same linkage groups as sex chromosomes.

Furthermore, it has been argued that hybridization between populations with different sex chromosome systems could promote sex chromosome turnover [45,145]. This is evidenced by a recent report of experimental crosses between a strain of the platyfish *Xiphophorus maculatus* with XY and the swordtail *Xiphophorus helleri* with ZW, which led to the translocation of the sex-determining region to an autosome in hybrids [145]. It is therefore reasonable to hypothesize that interaction between populations differing in their sex

chromosome systems [31,59,65,70,79,83,85–89] can give rise to novel sex chromosomes including multiple sex chromosome systems.

## 5. Conclusion

It is generally accepted that multiple sex chromosomes represent evolutionarily young sex chromosome systems, which can provide insight into sex chromosome evolution. This is seemingly less important in teleosts in which sex chromosome turnovers frequently occur. However, the multiple sex chromosomes could indicate the presence of homomorphic sex chromosomes within a lineage and, as we showed, allow for interesting inferences about the evolution of fish sex chromosomes. We compiled 440 cases of sex chromosomes in teleost fishes and collected detailed information about 75 cases of multiple sex chromosomes, which correspond to 60 independent origins of these systems. We further showed that multiple sex chromosomes are over-represented in the order Perciformes. Sex chromosome–autosome fusions were the most prevalent mechanism giving rise to multiple sex chromosomes in teleosts and their incidence was much higher in male-heterogametic than female-heterogametic taxa. We further documented a striking difference in patterns of differentiation of sex chromosomes between male and female heterogamety. We hypothesized that faster W sex chromosome differentiation may constrain sex chromosome turnover as it significantly reduces the time window in which it may take place. We also showed no significant association between the formation of multiple sex chromosomes and the percentage of uni-armed chromosomes in teleost karyotypes. We highlighted the gaps in research of multiple sex chromosomes in teleosts and emphasized the need for fine-scale analyses and broad sampling. Integration of molecular cytogenetics with genomics can fill the gaps and contribute to a more complex understanding of vertebrate sex chromosome evolution.

**Data accessibility.** All data and methods are provided in the electronic supplementary material.

**Authors’ contributions.** A.S. conceived the study and drafted the manuscript. P.N., M.d.B.C., M.A. and P.R. contributed to the conception and critically revised the manuscript. A.S. and M.A. compiled the datasets. A.S., M.A. and P.N. curated the datasets. M.F.P. performed the statistical analyses. M.d.B.C., M.F.P. and M.A. prepared the figures. All authors read and approved the final manuscript.

**Competing interests.** We declare we have no competing interests.

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- Bachtrog D. 2013 Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration. *Nat. Rev. Genet.* **14**, 113–124. (doi:10.1038/nrg3366)
- Stöck M *et al.* 2011 Ever-young sex chromosomes in European tree frogs. *PLoS Biol.* **9**, e1001062. (doi:10.1371/journal.pbio.1001062)
- Kamiya T *et al.* 2012 A trans-species missense SNP in *Amhr2* is associated with sex determination in the tiger pufferfish, *Takifugu rubripes* (fugu). *PLoS Genet.* **8**, e1002798. (doi:10.1371/journal.pgen.1002798)
- Zhou Q, Zhang J, Bachtrog D, An N, Huang Q, Jarvis ED, Gilbert MTP, Zhang G. 2014 Complex evolutionary trajectories of sex chromosomes across bird taxa. *Science* **346**, 1246338. (doi:10.1126/science.1246338)
- Cavoto E, Neuenschwander S, Goudet J, Perrin N. 2018 Sex-antagonistic genes, XY recombination and feminized Y chromosomes. *J. Evol. Biol.* **31**, 416–427. (doi:10.1111/jeb.13235)
- Nelson JS, Grande TC, Wilson MVH. 2016 *Fishes of the World*, 5th edn. Hoboken, NJ: John Wiley & Sons.
- Fricke R, Eschmeyer WN, Van der Laan R (eds). 2020 Eschmeyer's catalog of fishes: genera, species, references. See <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>. (electronic version accessed 1 November 2020).
- Devlin RH, Nagahama Y. 2002 Sex determination and sex differentiation in fish. *Aquaculture* **208**, 191–364. (doi:10.1016/S0044-8486(02)00057-1)
- Schartl M, Schmid M, Nanda I. 2016 Dynamics of vertebrate sex chromosome evolution: from equal size to giants and dwarfs. *Chromosoma* **125**, 553–571. (doi:10.1007/s00412-015-0569-y)
- Guiguen Y, Fostier A, Herpin A. 2019 Sex determination and differentiation in fish: genetic, genomic, and endocrine aspects. In *Sex control in aquaculture*, 1st edn (eds H-P Wang, F Piferrer, S-L Chen), pp. 35–64. Hoboken, NJ: John Wiley & Sons.
- Shen G, Wang P. 2019 Environmental sex determination and sex differentiation in teleosts – how sex is established. In *Sex control in aquaculture*, 1st edn (eds H-P Wang, F Piferrer, S-L Chen), pp. 85–116. Hoboken, NJ: John Wiley & Sons.
- Kuwamura T, Sunobe T, Sakai Y, Kadota T, Sawada K. 2020 Hermaphroditism in fishes: an annotated list of species, phylogeny, and mating system. *Ichthyol. Res.* **67**, 341–360. (doi:10.1007/s10228-020-00754-6)
- The Tree of Sex Consortium. 2014 Tree of sex: a database of sexual systems. *Sci. Data* **1**, 140015. (doi:10.1038/sdata.2014.15)
- Gamble T. 2016 Using RAD-seq to recognize sex-specific markers and sex chromosome systems. *Mol. Ecol.* **25**, 2114–2116. (doi:10.1111/mec.13648)
- Moreira-Filho O, Bertollo LAC, Galetti PM. 1993 Distribution of sex chromosome mechanisms in neotropical fish and description of a ZZ/ZW system in *Parodon hilarii* (Parodontidae). *Caryologia* **46**, 115–125. (doi:10.1080/00087114.1993.10797253)
- Schemberger MO, Bellafrente E, Nogaroto V, Almeida MC, Schühli GS, Artoni RF, Moreira-Filho O, Vicari MR. 2011 Differentiation of repetitive DNA sites and sex chromosome systems reveal closely related group in Parodontidae (Actinopterygii: Characiformes). *Genetica* **139**, 1499–1508. (doi:10.1007/s10709-012-9649-6)
- Cioffi MB, Moreira-Filho O, Almeida-Toledo LF, Bertollo LAC. 2012 The contrasting role of heterochromatin in the differentiation of sex chromosomes: an overview from Neotropical fishes. *J. Fish Biol.* **80**, 2125–2139. (doi:10.1111/j.1095-8649.2012.03272.x)
- Terencio ML, Schneider CH, Gross MC, Nogaroto V, de Almeida MC, Artoni RF, Vicari MR, Feldberg E. 2012 Repetitive sequences associated with differentiation of W chromosome in *Semaprochilodus taeniurus*. *Genetica* **140**, 505–512. (doi:10.1007/s10709-013-9699-4)
- Poltronieri J, Marquioni V, Bertollo LAC, Kejnovsky E, Molina WF, Liehr T, Cioffi MB. 2014 Comparative chromosomal mapping of microsatellites in *Leporinus* species (Characiformes, Anostomidae): unequal accumulation on the W chromosomes. *Cytogenet. Genome Res.* **142**, 40–45. (doi:10.1159/000355908)
- Yano CF, Bertollo LAC, Ezaz T, Trifonov V, Sember A, Liehr T, Cioffi MB. 2017 Highly conserved Z and molecularly diverged W chromosomes in the fish genus *Triporthus* (Characiformes, Triporthidae). *Heredity* **118**, 276–283. (doi:10.1038/hdy.2016.83)
- Mank JE, Avise JC. 2009 Evolutionary diversity and turn-over of sex determination in teleost fishes. *Sex. Dev.* **3**, 60–67. (doi:10.1159/000223071)
- Saunders PA. 2019 Sex chromosome turnovers in evolution. *eLS*. (doi:10.1002/9780470015902.a0028747)
- Vicoso B. 2019 Molecular and evolutionary dynamics of animal sex-chromosome turnover. *Nat. Ecol. Evol.* **3**, 1632–1641. (doi:10.1038/s41559-019-1050-8)
- Arai, R. 2011 *Fish karyotypes: a check list*, 1st edn. Tokyo, Japan: Springer.
- Palmer DH, Rogers TF, Dean R, Wright AE. 2019 How to identify sex chromosomes and their turnover. *Mol. Ecol.* **28**, 4709–4724. (doi:10.1111/mec.15245)
- Pan Q *et al.* 2019 Identification of the master sex determining gene in northern pike (*Esox lucius*) reveals restricted sex chromosome differentiation. *PLoS Genet.* **15**, e1008013. (doi:10.1371/journal.pgen.1008013)
- Kuhl H *et al.* 2021 A 180 My-old female-specific genome region in sturgeon reveals the oldest known vertebrate sex determining system with undifferentiated sex chromosomes. *Phil. Trans. R. Soc. B* **376**, 20200089. (doi:10.1098/rstb.2020.0089)
- de Oliveira RR, Feldberg E, Dos Anjos MB, Zuanon J. 2008 Occurrence of multiple sexual chromosomes (XX/X<sub>1</sub>Y<sub>2</sub> and Z<sub>1</sub>Z<sub>2</sub>Z<sub>3</sub>/Z<sub>1</sub>Z<sub>2</sub>W<sub>1</sub>W<sub>2</sub>) in catfishes of the genus *Ancistrus* (Siluriformes: Loricariidae) from the Amazon basin. *Genetica* **134**, 243–249. (doi:10.1007/s10709-007-9231-9)
- Kitano J, Peichel CL. 2012 Turnover of sex chromosomes and speciation in fishes. *Environ. Biol. Fishes* **94**, 549–558. (doi:10.1007/s10641-011-9853-8)
- Ferreira M, Garcia C, Matoso DA, de Jesus IS, Feldberg E. 2016 A new multiple sex chromosome system X<sub>1</sub>X<sub>2</sub>X<sub>3</sub>/X<sub>1</sub>Y<sub>1</sub>Y<sub>2</sub> in Siluriformes: cytogenetic characterization of *Bunocephalus coracoideus* (Aspredinidae). *Genetica* **144**, 591–599. (doi:10.1007/s10709-016-9927-9)
- Araya-Jaime C, Mateussi NTB, Utsunomia R, Costa-Silva GJ, Oliveira C, Foresti F. 2017 ZZ/ZO: the new system of sex chromosomes in *Eigenmannia* aff. *trilineata* (Teleostei: Gymnotiformes: Sternopygidae) characterized by molecular cytogenetics and DNA barcoding. *Zebrafish* **14**, 464–470. (doi:10.1089/zeb.2017.1422)
- Voff J-N, Schartl M. 2001 Variability of genetic sex determination in poeciliid fishes. *Genetica* **111**, 101–110. (doi:10.1023/A:1013795415808)
- Moore EC, Roberts RB. 2013 Polygenic sex determination. *Curr. Biol.* **23**, R510–R512. (doi:10.1016/j.cub.2013.04.004)
- Roberts NB, Juntti SA, Coyle KP, Dumont BL, Stanley MK, Ryan AQ, Fernald RD, Roberts RB. 2016 Polygenic sex determination in the cichlid fish *Astatotilapia burtoni*. *BMC Genomics* **17**, 835. (doi:10.1186/s12864-016-3177-1)
- White MJD. 1973 *Animal cytology & evolution*. Cambridge, UK: Cambridge University Press.
- Rens W *et al.* 2007 The multiple sex chromosomes of platypus and echidna are not completely identical and several share homology with the avian Z. *Genome Biol.* **8**, R243. (doi:10.1186/gb-2007-8-11-r243)
- Howell EC, Armstrong SJ, Filatov DA. 2009 Evolution of neo-sex chromosomes in *Silene diclinis*. *Genetics* **182**, 1109–1115. (doi:10.1534/genetics.109.103580)
- Giovannotti M *et al.* 2017 New insights into sex chromosome evolution in anole lizards (Reptilia, Dactyloidae). *Chromosoma* **126**, 245–260. (doi:10.1007/s00412-016-0585-6)
- Moreira-Filho O, Bertollo LAC, Galetti Jr PM. 1980 Evidences for a multiple sex chromosome system with female heterogamety in *Apareiodon affinis* (Pisces, Parodontidae). *Caryologia* **33**, 83–91. (doi:10.1080/00087114.1980.10796821)
- Toder R, O'Neill RJ, Wienberg J, O'Brien PC, Voullaire L, Graves JAM. 1997 Comparative chromosome painting between two marsupials: origins of an XX/X<sub>1</sub>Y<sub>2</sub> sex chromosome system. *Mamm. Genome* **8**, 418–422. (doi:10.1007/s00359900459)
- Pennell MW *et al.* 2015 Y fuse? Sex chromosome fusions in fishes and reptiles. *PLoS Genet.* **11**, e1005237. (doi:10.1371/journal.pgen.1005237)

42. Graves JAM. 2006 Sex chromosome specialization and degeneration in mammals. *Cell* **124**, 901–914. (doi:10.1016/j.cell.2006.02.024)
43. Graves JAM. 2014 Avian sex, sex chromosomes, and dosage compensation in the age of genomics. *Chromosome Res.* **22**, 45–57. (doi:10.1007/s10577-014-9409-9)
44. Pala I, Naurin S, Stervander M, Hasselquist D, Bensch S, Hansson B. 2012 Evidence of a neo-sex chromosome in birds. *Heredity* **108**, 264–272. (doi:10.1038/hdy.2011.70)
45. Graves JAM. 2016 Did sex chromosome turnover promote divergence of the major mammal groups? De novo sex chromosomes and drastic rearrangements may have posed reproductive barriers between monotremes, marsupials and placental mammals. *Bioessays* **38**, 734–743. (doi:10.1002/bies.201600019)
46. Kitano J *et al.* 2009 A role for a neo-sex chromosome in stickleback speciation. *Nature* **461**, 1079–1083. (doi:10.1038/nature08441)
47. Ross JA, Urton JR, Boland J, Shapiro MD, Peichel CL. 2009 Turnover of sex chromosomes in the stickleback fishes (Gasterosteidae). *PLoS Genet.* **5**, e1000391. (doi:10.1371/journal.pgen.1000391)
48. Betancur-R R *et al.* 2017 Phylogenetic classification of bony fishes. *BMC Evol. Biol.* **17**, 162. (doi:10.1186/s12862-017-0958-3)
49. Uyeno T, Miller RR. 1971 Multiple sex chromosomes in a Mexican cyprinodontid fish. *Nature* **231**, 452–453. (doi:10.1038/231452a0)
50. Levin B, Foster NR. 1972 Cytotaxonomic studies in Cyprinodontidae: multiple sex chromosomes in *Gambusia holbrooki*. *Not. Nat. Acad. Nat. Sci. Philadelphia* **446**, 1–5.
51. Sassi FM, Deon GA, Moreira-Filho O, Vicari VR, Bertollo LAC, Liehr T, de Oliveira EA, Cioffi MB. 2020 Multiple sex chromosomes and evolutionary relationships in Amazonian catfishes: the outstanding model of the genus *Harttia* (Siluriformes: Loricariidae). *Genes* **11**, 1179. (doi:10.3390/genes11101179)
52. de Oliveira EA *et al.* 2018 Tracking the evolutionary pathway of sex chromosomes among fishes: characterizing the unique XX<sub>1</sub>XY<sub>1</sub>Y<sub>2</sub> system in *Hoplias malabaricus* (Teleostei, Characiformes). *Chromosoma* **127**, 115–128. (doi:10.1007/s00412-017-0648-3)
53. Sember A, Bohlen J, Šlechtová V, Altmanová M, Symonová R, Ráb P. 2015 Karyotype differentiation in 19 species of river loach fishes (Nemacheilidae, Teleostei): extensive variability associated with rDNA and heterochromatin distribution and its phylogenetic and ecological interpretation. *BMC Evol. Biol.* **15**, 251. (doi:10.1186/s12862-015-0532-9)
54. Frolow SV. 1990 Differentiation of sex chromosomes in the Salmonidae. III. Multiple sex chromosomes in *Coregonus sardinella*. *Tsitologiya* **32**, 659–663.
55. Blanco DR *et al.* 2014 Origin of the X<sub>1</sub>X<sub>1</sub>X<sub>2</sub>X<sub>2</sub>/X<sub>1</sub>X<sub>2</sub>Y sex chromosome system of *Harttia punctata* (Siluriformes, Loricariidae) inferred from chromosome painting and FISH with ribosomal DNA markers. *Genetica* **142**, 119–126. (doi:10.1007/s10709-014-9759-4)
56. Ozouf-Costaz C, Hureau JC, Beaunier M. 1991 Chromosome studies on fish of the suborder Notothenioidei collected in the Weddell sea during Epos 3 cruise. *Cybio* **15**, 271–289.
57. Steinberg ER, Nieves M, Mudry MD. 2014 Multiple sex chromosome systems in howler monkeys (Platyrrhini, Alouatta). *Comp. Cytogenet.* **8**, 43–69. (doi:10.3897/CompCytogen.v8i1.6716)
58. Toups MA, Rodrigues N, Perrin N, Kirkpatrick M. 2019 A reciprocal translocation radically reshapes sex-linked inheritance in the common frog. *Mol. Ecol.* **28**, 1877–1889. (doi:10.1111/mec.14990)
59. Ferreira M *et al.* 2017 The *Bunocephalus coracoideus* species complex (Siluriformes, Aspredinidae). Signs of a speciation process through chromosomal, genetic and ecological diversity. *Front. Genet.* **8**, 120. (doi:10.3389/fgene.2017.00120)
60. Favarato RM, da Silva M, de Oliveira RR, Artoni RF, Feldberg E, Matoso DA. 2016 Cytogenetic diversity and the evolutionary dynamics of rDNA genes and telomeric sequences in the *Ancistrus* genus (Loricariidae: Ancistrini). *Zebrafish* **13**, 103–111. (doi:10.1089/zeb.2015.1140)
61. Parise-Maltempi PP, Martins C, Oliveira C, Foresti F. 2007 Identification of a new repetitive element in the sex chromosomes of *Leporinus elongatus* (Teleostei: Characiformes: Anostomidae): new insights into the sex chromosomes of *Leporinus*. *Cytogenet. Genome Res.* **116**, 218–223. (doi:10.1159/000098190)
62. Parise-Maltempi PP *et al.* 2013 Comparative analysis of sex chromosomes in *Leporinus* species (Teleostei, Characiformes) using chromosome painting. *BMC Genet.* **14**, 60. (doi:10.1186/1471-2156-14-60)
63. Nguyen P, Paladino LC. 2016 On the neo-sex chromosomes of Lepidoptera. In *Evolutionary biology* (ed. P. Pontarotti), pp. 171–185. Cham, Switzerland: Springer.
64. Ueno K, Ota K, Kobayashi T. 2001 Heteromorphic sex chromosomes of lizardfish (Synodontidae): focus on the ZZ-ZW<sub>1</sub>W<sub>2</sub> system in *Trachinocephalus myops*. *Genetica* **111**, 133–142. (doi:10.1023/A:1013749027620)
65. de Jesus CM, Bertollo LAC, Moreira-Filho O. 1999 Comparative cytogenetics in *Apareiodon affinis* (Pisces, Characiformes) and considerations regarding diversification of the group. *Genetica* **105**, 63–67. (doi:10.1023/A:1003592022927)
66. Mank JE, Promislow DEL, Avise JC. 2006 Evolution of alternative sex-determining mechanisms in teleost fishes. *Biol. J. Linn. Soc.* **87**, 83–93. (doi:10.1111/j.10958312.2006.00558.x)
67. da Cruz VP, Shimabukuro-Dias CK, Oliveira C, Foresti F. 2011 Karyotype description and evidence of multiple sex chromosome system X<sub>1</sub>X<sub>1</sub>X<sub>2</sub>X<sub>2</sub>/X<sub>1</sub>X<sub>2</sub>Y in *Potamotrygon* aff. *motoro* and *P. falkneri* (Chondrichthyes: Potamotrygonidae) in the upper Paraná River basin, Brazil. *Neotrop. Ichthyol.* **9**, 201–208. (doi:10.1590/S1679-62252011000100020)
68. Aichino DR, Pastori MC, Roncati HA, Ledesma MA, Swarça AC, Fenocchio AS. 2013 Characterization and description of a multiple sex chromosome system in *Potamotrygon motoro* (Chondrichthyes, Myliobatiformes) from the Paraná River, Argentina. *Genet. Mol. Res.* **12**, 2368–2375. (doi:10.4238/2013.January.16.3)
69. Ghigliotti L, Cheng CHC, Bonillo C, Coutanceau JP, Pisano E. 2013 *In situ* gene mapping of two genes supports independent evolution of sex chromosomes in cold-adapted Antarctic fish. *BioMed. Res. Int.* **2013**, 243938. (doi:10.1155/2013/243938)
70. Ghigliotti L, Cheng CC, Pisano E. 2016 Sex determination in Antarctic notothenioid fish: chromosomal clues and evolutionary hypotheses. *Polar Biol.* **39**, 11–22. (doi:10.1007/s00300-014-1601-z)
71. Kryanov E, Demidova T, Nagy B. 2016 Divergent karyotypes of the annual killifish genus *Nothobranchius* (Cyprinodontiformes, Nothobranchiidae). *Comp. Cytogenet.* **10**, 439–445. (doi:10.3897/CompCytogen.v10i3.9863)
72. Kryanov E, Demidova T. 2018 Extensive karyotype variability of African fish genus *Nothobranchius* (Cyprinodontiformes). *Comp. Cytogenet.* **12**, 387–402. (doi:10.3897/CompCytogen.v12i3.25092)
73. Jankun M, Ráb P, Vuorinen J. 1991 A karyotype study of vendace, *Coregonus albula* (Pisces, Coregoninae). *Hereditas* **115**, 291–294. (doi:10.1111/j.1601-5223.1992.tb00572.x)
74. Nugent CM, Easton AA, Norman JD, Ferguson MM, Danzmann RG. 2017 A SNP based linkage map of the Arctic charr (*Salvelinus alpinus*) genome provides insights into the diploidization process after whole genome duplication. *G3: Genes Genom. Genet.* **7**, 543–556. (doi:10.1534/g3.116.038026)
75. Blanco DR, Vicari MR, Lui RL, Bertollo LAC, Traldi JB, Moreira-Filho O. 2013 The role of the Robertsonian rearrangements in the origin of the XX<sub>1</sub>XY<sub>1</sub>Y<sub>2</sub> sex chromosome system and in the chromosomal differentiation in *Harttia* species (Siluriformes, Loricariidae). *Rev. Fish Biol. Fish.* **23**, 127–134. (doi:10.1007/s11160-012-9283-5)
76. Bertollo LAC, Mestriner CA. 1998 The X<sub>1</sub>X<sub>2</sub>Y sex chromosome system in the fish *Hoplias malabaricus*. II. Meiotic analyses. *Chromosome Res.* **6**, 141–147. (doi:10.1023/A:1009243114124)
77. Lisachov AP, Zadesenets KS, Rubtsov NB, Borodin PM. 2015 Sex chromosome synapsis and recombination in male guppies. *Zebrafish* **12**, 174–180. (doi:10.1089/zeb.2014.1000)
78. de Almeida-Toledo LF, Foresti F, Daniel MF, Toledo-Filho SA. 2000 Sex chromosome evolution in fish: the formation of the neo-Y chromosome in *Eigenmannia* (Gymnotiformes). *Chromosoma* **109**, 197–200. (doi:10.1007/s004120050428)
79. Caputo V, Machella N, Nisi-Cerioni P, Olmo E. 2001 Cytogenetics of nine species of Mediterranean blennies and additional evidence for an unusual multiple sex-chromosome system in *Parablennius tentacularis* (Perciformes, Blenniidae). *Chromosome Res.* **9**, 3–12. (doi:10.1023/A:1026779314932)
80. Cioffi MB, Bertollo LAC. 2010 Initial steps in XY chromosome differentiation in *Hoplias malabaricus*

- and the origin of an  $X_1X_2Y$  sex chromosome system in this fish group. *Heredity* **105**, 554–561. (doi:10.1038/hdy.2010.18)
81. Utsunomia R, Scacchetti PC, Pansonato-Alves JC, Oliveira C, Foresti F. 2014 Comparative chromosome mapping of U2 snRNA and 5S rRNA genes in *Gymnotus* species (Gymnotiformes, Gymnotidae): evolutionary dynamics and sex chromosome linkage in *G. pantanal*. *Cytogenet. Genome Res.* **142**, 286–292. (doi:10.1159/000362258)
  82. Bitencourt JA, Sampaio I, Ramos RTC, Vicari MR, Affonso PRAM. 2017 First report of sex chromosomes in Achiridae (Teleostei: Pleuronectiformes) with inferences about the origin of the multiple  $X_1X_2X_3/X_1X_2Y$  system and dispersal of ribosomal genes in *Achirus achirus*. *Zebrafish* **14**, 90–95. (doi:10.1089/zeb.2016.1333)
  83. Chen T-R, Reisman HM. 1970 A comparative chromosome study of the North American species of sticklebacks (Teleostei: Gasterosteidae). *Cytogenetics* **9**, 321–332. (doi.org/10.1159/000130102)
  84. Cioffi MB, Molina WF, Artoni RF, Bertollo LAC. 2012 Chromosomes as tools for discovering biodiversity – the case of Erythrinidae fish family. In *Recent trends in cytogenetic studies – methodologies and applications* (ed. P. Tirunilai), pp. 125–146. Rijeka, Croatia: InTech Publisher.
  85. Glugoski L, Deon G, Schott S, Vicari MR, Nogaroto V, Moreira-Filho O. 2020 Comparative cytogenetic analyses in *Ancistrus* species (Siluriformes: Loricariidae). *Neotrop. Ichthyol.* **18**, e200013. (doi:10.1590/1982-0224-2020-0013)
  86. Fernandes CA, Bailly D, Batista da Silva VF, Martins-Santos IC. 2010 System of multiple sex chromosomes in *Eigenmannia trilineata* López & Castello, 1966 (Sternopygidae, Gymnotiformes) from Iguatemi River Basin, MS, Brazil. *Cytologia* **75**, 463–466. (doi:10.1508/cytologia.75.463)
  87. Halačka K, Vetešník L, Lusk S, Mendel J, Papoušek I. 2007 The  $X_1X_2X_3/X_1X_2Y$  multiple sex chromosome system in the *Zingel zingel* (Pisces: Perciformes) from the Morava River (Czech Republic). *Caryologia* **60**, 222–225. (doi:10.1080/00087114.2007.10797940)
  88. Thorgaard GH. 1978 Sex chromosomes in the sockeye salmon: a Y-autosome fusion. *Can. J. Genet. Cytol.* **20**, 349–354. (doi:10.1139/g78-039)
  89. do Nascimento VD *et al.* 2018 Do multiple karyomorphs and population genetics of freshwater darter characines (*Apareiodon affinis*) indicate chromosomal speciation? *Zool. Anz.* **272**, 93–103. (doi:10.1016/j.jcz.2017.12.006)
  90. Prazdnikov DV, Vasil'ev VP, Vasil'eva ED. 2013 Polymorphism and interpopulation variability in karyotype of Caspian bighead goby *Neogobius gorlap* (Gobiidae, Perciformes). *J. Ichthyol.* **53**, 425–430. (doi:10.1134/S0032945213040073)
  91. Natri HM, Shikano T, Merilä J. 2013 Progressive recombination suppression and differentiation in recently evolved neo-sex chromosomes. *Mol. Biol. Evol.* **30**, 1131–1144. (doi:10.1093/molbev/mst035)
  92. de Almeida Toledo LF, Foresti F. 2001 Morphologically differentiated sex chromosomes in neotropical freshwater fish. *Genetica* **111**, 91–100. (doi:10.1023/A:1013768104422)
  93. Vicoso B, Emerson JJ, Zektser Y, Mahajan S, Bachtrog D. 2013 Comparative sex chromosome genomics in snakes: differentiation, evolutionary strata, and lack of global dosage compensation. *PLoS Biol.* **11**, e1001643. (doi:10.1371/journal.pbio.1001643)
  94. Sardell JM, Josephson MP, Dalziel AC, Peichel CL, Kirkpatrick M. 2020 Contrasting tempos of sex chromosome degeneration in sticklebacks. *BioRxiv.* (doi:10.1101/2020.09.17.300236)
  95. Yoshida K *et al.* 2014 Sex chromosome turnover contributes to genomic divergence between incipient stickleback species. *PLoS Genet.* **10**, e1004223. (doi:10.1371/journal.pgen.1004223)
  96. Yoshida K, Makino T, Kitano J. 2017 Accumulation of deleterious mutations on the neo-Y chromosome of Japan Sea stickleback (*Gasterosteus nipponicus*). *J. Hered.* **108**, 63–68. (doi:10.1093/jhered/esw054)
  97. Cai M *et al.* 2019 Chromosome assembly of *Collichthys lucidus*, a fish of Sciaenidae with a multiple sex chromosome system. *Sci. Data* **6**, 132. (doi:10.1038/s41597-019-0139-x)
  98. Xiao J *et al.* 2020 Development of a PCR-based genetic sex identification method in spinyhead croaker (*Collichthys lucidus*). *Aquaculture* **522**, 735130. (doi:10.1016/j.aquaculture.2020.735130)
  99. Peichel CL *et al.* 2020 Assembly of a young vertebrate Y chromosome reveals convergent signatures of sex chromosome evolution. *Genome Biol.* **21**, 177. (doi:10.1186/s13059-020-02097-x)
  100. Yano A *et al.* 2013 The sexually dimorphic on the Y-chromosome gene (*sdY*) is a conserved male-specific Y-chromosome sequence in many salmonids. *Evol. Appl.* **6**, 486–496. (doi:10.1111/eva.12032)
  101. Faber-Hammond J, Phillips RB, Park LK. 2012 The sockeye salmon neo-Y chromosome is a fusion between linkage groups orthologous to the coho Y chromosome and the long arm of rainbow trout chromosome 2. *Cytogenet. Genome Res.* **136**, 69–74. (doi:10.1159/000334583)
  102. Larson WA, McKinney GJ, Seeb JE, Seeb LW. 2016 Identification and characterization of sex-associated loci in sockeye salmon using genotyping-by-sequencing and comparison with a sex-determining assay based on the *sdY* gene. *J. Hered.* **107**, 559–566. (doi:10.1093/jhered/esw043)
  103. Cioffi MB, Molina WF, Moreira-Filho O, Bertollo LAC. 2011 Chromosomal distribution of repetitive DNA sequences highlights the independent differentiation of multiple sex chromosomes in two closely related fish species. *Cytogenet. Genome Res.* **134**, 295–302. (doi:10.1159/000329481)
  104. Ocalewicz K. 2013 Telomeres in fishes. *Cytogenet. Genome Res.* **141**, 114–125. (doi:10.1159/000354278)
  105. Zhang S, Zheng J, Zhang J, Wang Z, Wang Y, Cai M. 2018 Cytogenetic characterization and description of an  $X_1X_2X_3/X_1X_2Y$  sex chromosome system in *Collichthys lucidus* (Richardson, 1844). *Acta Oceanol. Sin.* **37**, 34–39. (doi:10.1007/s13131-018-1152-1)
  106. Šichová J, Ohno M, Dincă V, Watanabe M, Sahara K, Marec F. 2016 Fissions, fusions, and translocations shaped the karyotype and multiple sex chromosome constitution of the northeast-Asian wood white butterfly, *Leptidea amurensis*. *Biol. J. Linn. Soc.* **118**, 457–471. (doi:10.1111/bij.12756)
  107. Crepaldi C, Parise-Maltempi PP. 2020 Heteromorphic sex chromosomes and their DNA content in fish: an insight through satellite DNA accumulation in *Megaleporinus elongatus*. *Cytogenet. Genome Res.* **160**, 38–46 (doi:10.1159/000506265)
  108. Sember A, Bertollo LAC, Ráb P, Yano CF, Hatanaka T, de Oliveira EA, Cioffi MB. 2018 Sex chromosome evolution and genomic divergence in the fish *Hoplias malabaricus* (Characiformes, Erythrinidae). *Front. Genet.* **9**, 71. (doi:10.3389/fgene.2018.00071)
  109. de Moraes RLR *et al.* 2019 Comparative cytogenetics and neo-Y formation in small-sized fish species of the genus *Pyrrhulina* (Characiformes, Lebiastinidae). *Front. Genet.* **10**, 678. (doi:10.3389/fgene.2019.00678)
  110. Xu D *et al.* 2019 Deciphering the origin and evolution of the  $X_1X_2Y$  system in two closely-related *Oplegnathus* species (Oplegnathidae and Centrarchiformes). *Int. J. Mol. Sci.* **20**, 3571. (doi:10.3390/ijms20143571)
  111. Turelli M, Begunt DJ. 1997 Haldane's rule and X-chromosome size in *Drosophila*. *Genetics* **147**, 1799–1815. (doi:10.1093/genetics/147.4.1799)
  112. Bachtrog D, Mahajan S, Bracewell R. 2019 Massive gene amplification on a recently formed *Drosophila* Y-chromosome. *Nat. Ecol. Evol.* **3**, 1587–1597. (doi:10.1038/s41559-019-1009-9)
  113. Yoshida A *et al.* 2020 Evolution of multiple sex-chromosomes associated with dynamic genome reshuffling in *Leptidea* wood-white butterflies. *Heredity* **125**, 138–154. (doi:10.1038/s41437-020-0325-9)
  114. Nguyen P *et al.* 2013 Neo-sex chromosomes and adaptive potential in tortricid pests. *Proc. Natl Acad. Sci. USA* **110**, 6931–6936. (doi:10.1073/pnas.1220372110)
  115. Scacchetti PC, Utsunomia R, Pansonato-Alves JC. 2015 Repetitive DNA sequences and evolution of ZZ/ZW sex chromosomes in *Characidium* (Teleostei: Characiformes). *PLoS ONE* **10**, e0137231. (doi:10.1371/journal.pone.0137231)
  116. Pennell MW, Mank JE, Peichel CL. 2018 Transitions in sex determination and sex chromosomes across vertebrate species. *Mol. Ecol.* **27**, 3950–3963. (doi:10.1111/mec.14540)
  117. Sardell JM, Kirkpatrick M. 2020 Sex differences in the recombination landscape. *Am. Nat.* **195**, 361–379. (doi:10.1086/704943)
  118. Sardell JM, Cheng C, Dagilis AJ, Ishikawa A, Kitano J, Peichel CL, Kirkpatrick M. 2018 Sex differences in recombination in sticklebacks. *G3: Genes Genom. Genet.* **8**, 1971–1983. (doi:10.1534/g3.118.200166)
  119. Sutherland BJJ, Rico C, Audet C, Bernatchez L. 2017 Sex chromosome evolution, heterochiasmy, and physiological QTL in the salmonid brook charr *Salvelinus fontinalis*. *G3: Genes Genom. Genet.* **7**, 2749–2762. (doi:10.1534/g3.117.040915)
  120. Bergero R, Gardner J, Bader B, Yong L, Charlesworth D. 2019 Exaggerated heterochiasmy in a fish with

- sex-linked male coloration polymorphisms. *Proc. Natl Acad. Sci. USA* **116**, 6924–6931. (doi:10.1073/pnas.1818486116)
121. Butlin RK. 2005 Recombination and speciation. *Mol. Ecol.* **14**, 2621–2635. (doi:10.1111/j.1365-294X.2005.02617.x)
122. Hirst CE, Major AT, Smith CA. 2018 Sex determination and gonadal sex differentiation in the chicken model. *Int. J. Dev. Biol.* **62**, 153–166 (doi:10.1387/ijdb.170319cs)
123. Chen S *et al.* 2014 Whole-genome sequence of a flatfish provides insights into ZW sex chromosome evolution and adaptation to a benthic lifestyle. *Nat. Genet.* **46**, 253–260. (doi:10.1038/ng.2890)
124. Cui Z *et al.* 2017 Genome editing reveals *dmrt1* as an essential male sex-determining gene in Chinese tongue sole (*Cynoglossus semilaevis*). *Sci. Rep.* **7**, 42213. (doi:10.1038/srep42213)
125. Tomaszewicz M, Medvedev P, Makova KD. 2017 Y and W chromosome assemblies: approaches and discoveries. *Trends Genet.* **33**, 266–282. (doi:10.1016/j.tig.2017.01.008)
126. van Doorn GS, Kirkpatrick M. 2007 Turnover of sex chromosomes induced by sexual conflict. *Nature* **449**, 909–912. (doi:10.1038/nature06178)
127. Blaser O, Grossen C, Neuenschwander S, Perrin N. 2013 Sex-chromosome turnovers induced by deleterious mutation load. *Evolution* **67**, 635–645. (doi:10.1111/j.1558-5646.2012.01810.x)
128. Pokorná M, Altmanová M, Kratochvíl L. 2014 Multiple sex chromosomes in the light of female meiotic drive in amniote vertebrates. *Chromosome Res.* **22**, 35–44. (doi:10.1007/s10577-014-9403-2)
129. Kirkpatrick M. 2017 The evolution of genome structure by natural and sexual selection. *J. Hered.* **108**, 3–11. (doi:10.1093/jhered/esw041)
130. Carabajal PLZ *et al.* 2019 Sex chromosome turnover in moths of the diverse superfamily Gelechioidea. *Genome Biol. Evol.* **11**, 1307–1319. (doi:10.1093/gbe/evz075)
131. Soares RX, Bertollo LAC, Cioffi MB, Costa GWWF, Molina WF. 2014 Chromosomal distribution of two multigene families and the unusual occurrence of an  $X_1X_1X_2X_2/X_1X_2Y$  sex chromosome system in the dolphinfish (Coryphaenidae): an evolutionary perspective. *Genet. Mol. Res.* **13**, 2470–2479. (doi:10.4238/2014.April.3.19)
132. de Villena FPM, Sapienza C. 2001 Female meiosis drives karyotypic evolution in mammals. *Genetics* **159**, 1179–1189. (doi:10.1093/genetics/159.3.1179)
133. Yoshida K, Kitano J. 2012 The contribution of female meiotic drive to the evolution of neo-sex chromosomes. *Evolution* **66**, 3198–3208. (doi:10.1111/j.1558-5646.2012.01681.x)
134. O'Meally D, Ezaz T, Georges A, Sarre SD, Graves JAM. 2012 Are some chromosomes particularly good at sex? Insights from amniotes. *Chromosome Res.* **20**, 7–19. (doi:10.1007/s10577-011-9266-8)
135. Ross JA, Peichel CL. 2008 Molecular cytogenetic evidence of rearrangements on the Y chromosome of the threespine stickleback fish. *Genetics* **179**, 2173–2182. (doi:10.1534/genetics.108.088559)
136. Henning F, Trifonov V, Ferguson-Smith MA, de Almeida-Toledo LF. 2008 Non-homologous sex chromosomes in two species of the genus *Eigenmannia* (Teleostei: Gymnotiformes). *Cytogenet. Genome Res.* **121**, 55–58. (doi:10.1159/000124382)
137. Cioffi MB, Yano CF, Sember A, Bertollo LAC. 2017 Chromosomal evolution in lower vertebrates: sex chromosomes in Neotropical fishes. *Genes* **8**, 258. (doi:10.3390/genes8100258)
138. Henning F, Moysés CB, Calcagnotto D, Meyer A, de Almeida-Toledo LF. 2011 Independent fusions and recent origins of sex chromosomes in the evolution and diversification of glass knife fishes (*Eigenmannia*). *Heredity* **106**, 391–400. (doi:10.1038/hdy.2010.82)
139. Charlesworth D, Charlesworth B. 1980 Sex differences in fitness and selection for centric fusions between sex-chromosomes and autosomes. *Genet. Res. Camb.* **35**, 205–214. (doi:10.1017/S0016672300014051)
140. Matsumoto T, Kitano J. 2016 The intricate relationship between sexually antagonistic selection and the evolution of sex chromosome fusions. *J. Theor. Biol.* **404**, 97–108. (doi:10.1016/j.jtbi.2016.05.036)
141. Jeffries DL, Gerchen JF, Scharmann M, Pannell J. 2021 A neutral model for the loss of recombination on sex chromosomes. *Phil. Trans. R. Soc. B* **376**, 20200096. (doi:10.1098/rstb.2020.0096)
142. Perrin N. 2021 Sex-chromosome evolution in frogs: what role for sex-antagonistic genes? *Phil. Trans. R. Soc. B* **376**, 20200094. (doi:10.1098/rstb.2020.0094)
143. Marshall Graves JA, Peichel CL. 2010 Are homologies in vertebrate sex determination due to shared ancestry or to limited options? *Genome Biol.* **11**, 205. (doi:10.1186/gb-2010-11-4-205)
144. Pan Q, Kay T, Depincé A, Adolphi M, Scharl M, Guiguen Y, Herpin A. 2021 Evolution of master sex determiners: TGF- $\beta$  signalling pathways at regulatory crossroads. *Phil. Trans. R. Soc. B* **376**, 20200091. (doi:10.1098/rstb.2020.0091)
145. Franchini P *et al.* 2018 Long-term experimental hybridisation results in the evolution of a new sex chromosome in swordtail fish. *Nat. Commun.* **9**, 5136. (doi:10.1038/s41467-018-07648-2)