

Turnover of sex chromosomes and speciation in fishes

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Abstract Closely related species of fishes often have different sex chromosome systems. Such rapid turnover of sex chromosomes can occur by several mechanisms, including fusions between an existing sex chromosome and an autosome. These fusions can result in a multiple sex chromosome system, where a species has both an ancestral and a neo-sex chromosome. Although this type of multiple sex chromosome system has been found in many fishes, little is known about the mechanisms that select for the formation of neo-sex chromosomes, or the role of neo-sex chromosomes in phenotypic evolution and speciation. The identification of closely related, sympatric species pairs in which one species has a multiple sex chromosome system and the other has a simple sex chromosome system provides an opportunity to study sex chromosome turnover. Recently, we found that a population of threespine stickleback (*Gasterosteus aculeatus*) from Japan has an X_1X_2Y multiple sex chromosome system resulting from a fusion between the ancestral Y chromosome and an autosome, while a sympatric

threespine stickleback population has a simple XY sex chromosome system. Furthermore, we demonstrated that the neo-X chromosome (X_2) plays an important role in phenotypic divergence and reproductive isolation between these sympatric stickleback species pairs. Here, we review multiple sex chromosome systems in fishes, as well as recent advances in our understanding of the evolutionary role of sex chromosome turnover in stickleback speciation.

Keywords Multiple sex chromosomes · Neo-sex chromosome · X_1X_2Y · Stickleback · Sexual conflict · Speciation

Turnover of sex chromosome in fishes

Sex chromosomes have repeatedly and independently evolved in plant and animal species with genetic sex-determination mechanisms (Bull 1983). Although some taxonomic groups appear to have stable sex chromosomes that have been conserved across hundreds of millions of years of evolution, there are many examples of sex chromosome turnover, where even closely related species have different sex chromosomes (White 1973). Heteromorphic sex chromosomes have been found in about 10% of fish species karyotyped (Devlin and Nagahama 2002), and many independent groups of fishes show evidence for sex chromosome turnover (Devlin and Nagahama

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2002; Mank et al. 2006). In fishes, there is evidence that sex chromosome turnover has occurred through multiple mechanisms, including the transposition of an existing male-determination locus to an autosome (Woram et al. 2003), the evolution of a new male-determination locus on an autosome (Kondo et al. 2006; Tanaka et al. 2007), and fusions between an autosome and an existing Y-chromosome (Kitano et al. 2009; Ross et al. 2009). In this review, we focus on the evolution of sex chromosome-autosome fusions, which appear to be quite common in fishes. Then, we discuss the mechanisms that might select for these fusions as well as their potential role in phenotypic evolution and speciation.

Multiple sex chromosome systems in fishes

Most species with sex chromosomes either have a simple male heteromorphic (XX female/XY male) or female heteromorphic (ZW female/ZZ male) system. Fusions between an autosome and a sex chromosome (X, Y, Z, or W) create what is commonly referred to as a multiple sex chromosome system. For example, when an autosome fuses to a Y chromosome, this creates an X_1X_2Y sex chromosome system (White 1973). In this case, males have one neo-Y chromosome, one ancestral X chromosome (X_1), and one neo-X chromosome (X_2), while females have two pairs of X chromosomes ($X_1X_1X_2X_2$) (Fig. 1). Similarly, fusions between an

autosome and an X, Z, or W chromosome will create an XY_1Y_2 , ZW_1W_2 , or Z_1Z_2W system, respectively. All of these types of systems have been found in a variety of taxonomic groups. The first multiple sex chromosome system identified in fishes was found by Uyeno and Miller (1971) in a species of Mexican killifish; males have an X_1X_2Y sex chromosome system, with one large metacentric Y chromosome that is missing in females and two small acrocentric X chromosomes that are also present in females (Uyeno and Miller 1971). Since then, multiple sex chromosome systems have been identified in many fish species across diverse families (Table 1). The presence of multiple sex chromosome systems in diverse groups of fishes suggests that they have evolved independently in multiple lineages.

Interestingly, X_1X_2Y systems are more common in fishes than the other types of multiple sex chromosome systems (35/41) (Table 2). X_1X_2Y systems can arise not only through fusions between an autosome and a Y chromosome, but also through centric fission of the X chromosome in species with an XY system or through reciprocal translocations between the X chromosome and an autosome in species with an ancestral XX female/XO male sex chromosome system (Fig. 1) (White 1973). Several lines of evidence support the idea that the multiple sex chromosome systems in fishes arose mainly through fusions between a Y chromosome and an autosome. First, in many species, the neo-Y

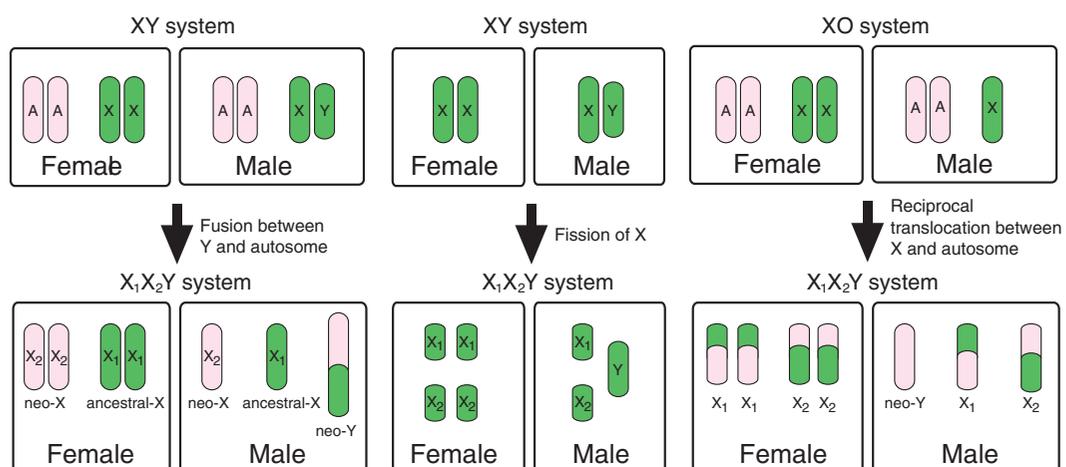


Fig. 1 Three proposed mechanisms for the formation of an X_1X_2Y multiple sex chromosome system, based on White (1983). Autosome, X chromosome, ancestral-X chromosome,

neo-X chromosome, and Y chromosome are abbreviated as A , X , X_1 , X_2 , and Y , respectively

Table 1 List of fishes that have multiple sex chromosome systems

Order	Family	Genus	Species	2n (F/M)	Comments ^a	References
$X_1X_1X_2X_2/X_1X_2Y$						
Anguilliformes	Ophichthidae	<i>Muraenichthys</i>	<i>gymnotus</i>	48/47	Y-A fusion	(Murofushi and Yosida 1984)
Beryciformes	Berycidae	<i>Beryx</i>	<i>splendens</i>	48/47	Y-A fusion	(Ojima and Kikuno 1986)
Characiformes	Erythrinidae	<i>Erythrinus</i>	<i>erythrinus B^b</i>	54/53	Y-A fusion; <i>E. erythrinus</i> A population has no obvious sex chromosome (2n=54)	(Bertollo et al. 2004)
Characiformes	Erythrinidae	<i>Erythrinus</i>	<i>erythrinus C^b</i>	52/51	Y-A fusion; <i>E. erythrinus</i> A population has no obvious sex chromosome (2n=54)	(Bertollo et al. 2004)
Characiformes	Erythrinidae	<i>Erythrinus</i>	<i>erythrinus D^b</i>	52/51	Y-A fusion; <i>E. erythrinus</i> A population has no obvious sex chromosome (2n=54)	(Bertollo et al. 2004)
Characiformes	Erythrinidae	<i>Hoplias</i>	<i>malabaricus</i>	40/39	Y-A fusion; other <i>H. malabaricus</i> karyomorphs are XXXY (2n=42) or XXXY ₁ Y ₂ (2n=41/40)	(Bertollo et al. 2000; Rosa et al. 2009)
Clupeiformes	Clupeidae	<i>Brevoortia</i>	<i>aurea</i>	46/45	Y-A fusion; <i>B. pectinata</i> has no obvious sex chromosome (2n=46)	(Brum and Galetti 1992)
Cypriniformes	Cobitidae	<i>Cobitis</i>	<i>taenia striata</i>	50/49	Y-A fusion	(Saitoh 1989)
Cyprinodontiformes	Cyprinodontidae	<i>Garmanella</i>	<i>pulchra</i>	50/49	Y-A fusion	(Levin and Foster 1972)
Cyprinodontiformes	Cyprinodontidae	<i>Megapsilon</i>	<i>aporus</i>	48/47	first Y-A fusion identified in fishes	(Uyeno and Miller 1971)
Cyprinodontiformes	Goodeidae	<i>Allodontichthys</i>	<i>hubbsi</i>	42/41	Y-A fusion; no obvious sex chromosomes in family (2n=48)	(Uyeno et al. 1983)
Cyprinodontiformes	Nothobranchiidae	<i>Nothobranchius</i>	<i>guentheri</i>	36/35	Y-A fusion	(Ewulonu et al. 1985)
Gasterosteiformes	Gasterosteidae	<i>Gasterosteus</i>	<i>aculeatus (JS)</i>	42/41	Y-A fusion; sympatric <i>G. aculeatus</i> population is XXXY (2n=42)	(Kitano et al. 2009)
Gasterosteiformes	Gasterosteidae	<i>Gasterosteus</i>	<i>wheatlandi</i>	42/41	Y-A fusion; closely related, sympatric species <i>G. aculeatus</i> is XXXY (2n=42)	(Ross et al. 2009)
Gymnotiformes	Gymnotidae	<i>Gymnotus</i>	<i>pantanal</i>	40/39	Y-A fusion; two closely related, sympatric species have no obvious sex chromosomes (2n=40 or 54)	(Margarido et al. 2007)
Gymnotiformes	Hypopomidae	<i>Brachyhypopomus</i>	<i>pinnicaudatus</i>	42/41	Y-A fusion	(Almeida-Toledo et al. 2000a)
Gymnotiformes	Stemopygidae	<i>Eigenmannia</i>	sp.2	32/31	Y-A fusion; closely related, sympatric species <i>E. virescens</i> is XXXY (2n=38)	(Almeida-Toledo et al. 2000b; Almeida-Toledo et al. 1984)
Perciformes	Blenniidae	<i>Parablennius</i>	<i>tentacularis</i>	48/47	Y-A fusion; closely related species have no obvious sex chromosome	(Caputo et al. 2001)
Perciformes	Callionymidae	<i>Callionymus</i>	<i>bonitiguri</i>	38/37	Y-A fusion; closely related species <i>C. punctatus</i> and <i>C. dorysuss</i> are XXXY	(Murofushi et al. 1983)
Perciformes	Callionymidae	<i>Callionymus</i>	<i>ornatipinnis</i>	38/37	Y-A fusion; closely related species <i>C. punctatus</i> and <i>C. dorysuss</i> are XXXY	(Murofushi et al. 1983)
Perciformes	Channichthyidae	<i>Chaenodraco</i>	<i>wilsoni</i>	48/47	Y-A fusion; closely related species have no obvious sex chromosome	(Morescalchi et al. 1992)
Perciformes	Channichthyidae	<i>Chionodraco</i>	<i>hamatus</i>	48/47	Y-A fusion; closely related species have no obvious sex chromosome	(Morescalchi et al. 1992)
Perciformes	Channichthyidae	<i>Chionodraco</i>	<i>myersi</i>	48/47	Y-A fusion; closely related species have no obvious sex chromosome	(Morescalchi et al. 1992)

Table 1 (continued)

Order	Family	Genus	Species	2n (F/M)	Comments ^a	References
Perciformes	Channichthyidae	<i>Chionobathyscus</i>	<i>dewitti</i>	48/47	Y-A fusion; closely related species have no obvious sex chromosome	(Morescalchi et al. 1992)
Perciformes	Channichthyidae	<i>Pagetopsis</i>	<i>macropterus</i>	48/47	Y-A fusion; closely related species have no obvious sex chromosome	(Morescalchi et al. 1992)
Perciformes	Eleotrididae	<i>Awavous</i>	<i>strigatus</i>	ND	Species in same family <i>Dormitator maculatus</i> is XX/XY	(Oliveira and Almeida-Toledo 2006)
Perciformes	Gobiidae	<i>Ctenogobius</i>	<i>shufeldti</i>	48/47	Y-A fusion; family has XX/XY, ZW/ZZ and XX/XO sex chromosome systems	(Pezold 1984)
Perciformes	Lutjanidae	<i>Lutjanus</i>	<i>quinquefasciatus</i>	48/47	Y-A fusion; closely related species <i>L. kasmira</i> has no obvious sex chromosome (2n=48)	(Ueno and Takai 2008)
Perciformes	Monodactylidae	<i>Monodactylus</i>	<i>sebae</i>	48/47	Y-A fusion; closely related species <i>M. argenteus</i> has no obvious sex chromosome (2n=48)	(Suzuki et al. 1988)
Perciformes	Percidae	<i>Zingel</i>	<i>zingel</i>	48/47	Y-A fusion; other species in family are XX/XY	(Halacka et al. 2007)
Salmoniformes	Salmonidae	<i>Oncorhynchus</i>	<i>nerka</i>	58/57	Y-A fusion; closely related species <i>O. mykiss</i> is XX/XY	(Thorgaard 1978; Ueda and Ojima 1984)
Scorpaeniformes	Tetraoideae	<i>Hypodytes</i>	<i>rubripinnis</i>	48/47	Y-A fusion	(Ueno and Kang 1992)
Siluriformes	Siluridae	<i>Ompok</i>	<i>bimaculatus</i>	42/41	Y-A fusion	(Rishi 1976)
Tetraodontiformes	Monacanthidae	<i>Stephanolepis</i>	<i>cirrhifer</i>	34/33	Y-A fusion	(Murofushi et al. 1980)
Tetraodontiformes XXXY ₁ Y ₂	Monacanthidae	<i>Stephanolepis</i>	<i>hispidus</i>	34/33	Y-A fusion	(Brum 1996)
Characiformes	Erythrinidae	<i>Hoplias</i>	<i>malabaricus</i>	40/41	Other <i>H. malabaricus</i> karyomorphs are XX/XY or XX/X ₁ X ₂ Y	(Bertollo et al. 2000; Bertollo et al. 1983)
Siluriformes	Loricariidae	<i>Ancistrus</i>	<i>sp.1</i>	38/39	Family also has XX/XY, ZW/ZZ, and XX/XO sex chromosome systems	(Alves et al. 2006; Oliveira et al. 2008)
Siluriformes	Loricariidae	<i>Harttia</i>	<i>carvalhoi</i>	52/53	Closely related species do not have obvious sex chromosomes	(Centofante et al. 2006)
Z ₁ Z ₂ W ₁ W ₂ Z ₁ Z ₂ Z ₂	Siluriformes	<i>Ancistrus</i>	<i>sp.2</i>	52/52	Family also has XX/XY, ZW/ZZ, and XX/XO sex chromosome systems	(Alves et al. 2006; Oliveira et al. 2008)
ZW ₁ W ₂ /ZZ	Aulopiformes	<i>Trachinocephalus</i>	<i>myops</i>	27/26	Z-A fusion; other species in family are ZW/ZZ (2n=48)	(Ueno et al. 2001)
Characiformes	Parodontidae	<i>Apareiodon</i>	<i>affinis</i>	55/54	Fission of W; sympatric population has no obvious sex chromosome (2n=54)	(Jesus et al. 1999; Moreira-Filho et al. 1980)

^a When mechanisms creating the neo sex chromosomes are suggested in the original paper, they are noted

^b These three karyomorphs are presented as different species in this table, but might constitute a single species

ND=no data available

Table 2 Number of cases of different multiple sex chromosome systems found in each order

	$X_1X_1X_2X_2/$ X_1X_2Y	$XX/$ XY_1Y_2	$Z_1Z_2W_1W_2/$ $Z_1Z_2Z_1Z_2$	$Z W_1W_2/$ ZZ
Anguilliformes	1			
Aulopiformes				1
Beryciformes	1			
Characiformes	4 ^a	1		1
Clupeiformes	1			
Cypriniformes	1			
Cyprinodontiformes	4			
Gasterosteiformes	2			
Gymnotiformes	3			
Perciformes	13			
Salmoniformes	1			
Scorpaeniformes	1			
Siluriformes	1	2	1	
Tetraodontiformes	2			

^a Three of these examples are karyomorphs, which might constitute a single species

chromosome is a large metacentric chromosome, while the two X chromosomes are small acrocentric chromosomes, suggesting that the neo-Y chromosome arose through a Robertsonian fusion of two acrocentric chromosomes (Ueno and Takai 2008). Consistent with this hypothesis, the neo-Y is associated with both the X_1 and the X_2 chromosomes during meiosis in all species examined (Ueno and Takai 2008).

Second, XX/XO systems are not as common as XY system in fish, with only twelve examples of XX/XO systems documented to date (Devlin and Nagahama 2002; Alves et al. 2006). Furthermore, only one of these species (*Gobiodon citrinus*) is found in a family (Gobiidae) that also contains a species (*Ctenogobius shufeldti*) with an X_1X_2Y system (Pezold 1984). In seven of the known X_1X_2Y systems, a closely related species in the same genus or other populations of the same species have an XX/XY sex chromosome system, suggesting that this may be the ancestral state (Table 1). In three of these cases, cytogenetic evidence demonstrates that the ancestral Y has indeed fused to an autosome (Kitano et al. 2009; Ross et al. 2009; Cioffi and Bertollo 2010). Although no evidence for heteromorphic sex chromosomes has been found in eleven cases where closely related species have been analyzed cytogenetically (Table 1), it is possible that

these species do have an XX/XY sex chromosome system that cannot be distinguished using traditional cytogenetic tools. For example, it was originally reported that the threespine stickleback (*Gasterosteus aculeatus*) had no heteromorphic sex chromosomes, but later studies using fluorescence in situ hybridization revealed that the X and the Y can be distinguished (Ross and Peichel 2008).

Finally, although centric fission of the X chromosome can result in the formation of an X_1X_2Y system (Fig. 1), this is unlikely to be the cause in most fish species with this system. When fissions create an X_1X_2Y system, closely related species with an XY system are expected to have fewer chromosome pairs (2n) than the species with an X_1X_2Y system. However, there are no such cases (Table 1).

Thus, it is likely that most of the X_1X_2Y sex chromosome systems in fishes are derived from fusions between an existing Y chromosome (whether cytogenetically visible or not) and an autosome. However, it is still unknown why these Y-autosome fusions are relatively common in fishes, and whether they have any role in phenotypic evolution or speciation. In order to address these questions, it is particularly useful to identify extant species pairs in which one species has the ancestral simple sex chromosome and the other has the derived multiple sex chromosome system. Indeed, for a few of the species with multiple sex chromosome systems listed in Table 1, such species pairs can be found in sympatry or parapatry. For example, in a single watershed, there are sympatric populations of *Hoplias* aff. *malabaricus*; some populations have a simple XY system, other populations have an X_1X_2Y system, and still others have a XY_1Y_2 system (Bertollo et al. 1983; Bertollo et al. 2000; Rosa et al. 2009). Similarly, two overlapping sub-species of *Eigenmannia* have non-homologous sex chromosomes; *E. virescens* has an XY system and *E. sp.2* has an X_1X_2Y system (Almeida-Toledo et al. 1984; Almeida-Toledo et al. 2000b; Henning et al. 2008). In eastern Hokkaido, Japan, an incipient species of threespine stickleback (*G. aculeatus*) has an X_1X_2Y system; this Japan Sea form is sympatric with the ancestral Pacific Ocean form, which has a simple XY system (Kitano et al. 2009). In the Japan Sea form, cytogenetic studies and linkage analysis revealed that the X_1X_2Y system results from a fusion between an autosome (chromosome 9) and the ancestral Y chromosome

(chromosome 19) (Kitano et al. 2009). A similar fusion has occurred in the blackspotted stickleback (*G. wheatlandi*), which is sympatric with the Atlantic Ocean population of threespine stickleback on the eastern coast of North America (FitzGerald and Wootton 1993). Like the Japan Sea form, *G. wheatlandi* also has an X_1X_2Y system, but chromosome 12 has fused to the ancestral Y chromosome (Ross et al. 2009). These data suggest that the fusions have occurred independently in the two stickleback lineages.

What drives sex chromosome turnover?

Theoretical work has suggested that the presence of genes with sexually antagonistic effects (i.e. genes with alleles that have differential fitness effects in males and females) on an ancestral autosome might drive the turnover of sex chromosomes (Charlesworth and Charlesworth 1980; van Doorn and Kirkpatrick 2007). Alleles that enhance fitness in one sex will not necessarily increase, and might even reduce, the fitness of the other sex. If such alleles with sexually antagonistic effects are present on autosomes, they will not easily spread within a population, because selection for an increase in allele frequency in one sex will be counteracted by selection against the allele in the other sex. However, if a sexually antagonistic gene is localized on a sex chromosome and tightly linked to the sex-determination locus, both males and females can increase their fitness by having different allele frequencies at the sexually antagonistic locus. Thus, the presence of genes with sexually antagonistic effects on an autosome might select for the evolution of a new sex-determination locus on that autosome, the transposition of an existing sex-determination locus to that autosome, or the fusion of that autosome to an existing sex chromosome.

Although empirically testing this hypothesis presents a major challenge, the identification of closely related species with different sex chromosome systems provides a unique opportunity to test whether sexual antagonism plays a role in sex chromosome turnover. For example, a new female-determination locus has evolved in species of Lake Malawi cichlids, and a gene involved in sexually dimorphic pigmentation is tightly linked to the female sex-determination locus on the invading W chromosome (Roberts et al. 2009). Because the sexually

dimorphic pigmentation pattern may be beneficial in females but detrimental in males of these species, sexually antagonistic selection might have played an important role in the evolution of this new ZW sex chromosome system.

Similarly, we have some preliminary evidence that sexually antagonistic traits are found on the neo-X chromosome created by the Y-autosome fusion in the Japan Sea threespine stickleback. Dorsal spine length is sexually dimorphic in the Japan Sea population (Kitano et al. 2007b) and has been suggested as a sexually antagonistic trait in sticklebacks (Reimchen and Nosil 2004). Because variation in dorsal spine length maps to the Japan Sea neo-X chromosome (Kitano et al. 2009), it is possible that the presence of sexually antagonistic variation for spine length on an ancestral autosome might have selected for the fixation of the sex chromosome fusion. However, we have not yet confirmed that spine length is a sexually antagonistic trait or identified any genes with sexually antagonistic alleles on the ancestral autosome (chromosome 9) of the Pacific Ocean population. Further comparative genomic and functional studies of genes on chromosome 9 in the ancestral Pacific Ocean and the derived Japan Sea sticklebacks will provide insights into whether sexually antagonistic selection plays an important role in sex chromosome turnover.

Role of sex chromosome turnover in speciation

It has long been appreciated that sex chromosomes might play a special role in speciation. There is abundant empirical evidence demonstrating that hybrid sterility genes are preferentially localized on sex chromosomes (Coyne and Orr 2004; Presgraves 2008; Qvarnstrom and Bailey 2009). Such an overrepresentation of hybrid sterility genes on the sex chromosomes may be due to multiple factors, such as faster X evolution, accumulation of sexually antagonistic genes, sex ratio meiotic drive, transcriptional suppression of sex chromosomes during early spermatogenesis, and gene traffic between the X and autosomes (Coyne and Orr 2004; Presgraves 2008; Moyle et al. 2010). The relative role of the sex chromosomes in other isolating barriers is still debated (Qvarnstrom and Bailey 2009), but there are empirical data indicating that the sex chromosomes harbor genes

that are important for behavioral isolation between closely related species (Prowell 1998; Reinhold 1998; Lindholm and Breden 2002).

Although there is good evidence that the sex chromosomes do play an important role in speciation, the role of sex chromosome turnover in speciation has been less appreciated. However, genes that play a role in reproductive isolation between populations might accumulate on neo-sex chromosomes for many of the same reasons that they are found on ancestral sex chromosomes. In this case, sex chromosome turnover might actually promote speciation. We have tested this idea by performing genetic linkage analysis of the isolating barriers present between the Japan Sea form (X_1X_2Y) and the Pacific Ocean form (XY) of threespine sticklebacks. These two forms diverged during a period of geographical isolation between the Sea of Japan and the Pacific Ocean about 1.5–2 million years ago. Currently, they are sympatric in eastern Hokkaido, but are reproductively isolated with a low level of hybridization (Higuchi and Goto 1996; Yamada et al. 2001; Kitano et al. 2007a; Yamada et al. 2007). Reproductive isolation between this stickleback species pair results from multiple isolating barriers, including ecogeographical isolation (Kume et al. 2005; Kume et al. 2010), seasonal isolation (Kume et al. 2005), behavioral isolation (Kitano et al. 2007a), reduced fitness of hybrids (Kitano et al. 2009), and hybrid male sterility (Kitano et al. 2007a).

Although the male hybrids resulting from a cross between a Japan Sea female and a Pacific Ocean male are sterile, hybrid males resulting from crosses in the opposite direction and all hybrid females are fertile (Kitano et al. 2007a). Therefore, we were able to make artificial crosses between these two sympatric species and utilize the numerous genomic and genetic tools available for sticklebacks (Kingsley and Peichel 2007) to conduct quantitative trait locus (QTL) mapping of isolating barriers between the species pair (Kitano et al. 2009). Our QTL mapping revealed that hybrid sterility and male courtship display traits mapped to the ancestral X chromosome (chromosome 19) and the neo-X chromosome (chromosome 9) respectively (Kitano et al. 2009). Thus, a neo-X chromosome resulting from a fusion between the ancestral Y chromosome and an autosome contains genes important for the evolution of male courtship displays and can contribute to reproductive isolation

between sympatric stickleback species. This is consistent with the idea that the turnover of sex chromosome might promote speciation.

Conclusions

Species with multiple sex chromosome systems are prevalent in fishes. In some cases, they can be found in sympatry or parapatry with closely related species that have the ancestral, simple sex chromosome system. These species pairs provide a unique opportunity to gain insight into the evolutionary forces that drive the evolution of new sex chromosomes and to the consequences of their evolution on phenotypic divergence and speciation. First, by identifying the genomic loci important for reproductive isolation or phenotypic divergence between closely related species, we can investigate whether the new sex chromosomes created by fusions can contribute to speciation, as we found in the Japanese species pair. Because the gaps between model and non-model organisms are decreasing with the advent of high-throughput sequencing technologies (Rokas and Abbot 2009), it should be possible to conduct similar genetic and genomic analyses on other fish species pairs to investigate the generality of our findings. Second, these systems could be used to test the hypothesis that sexual conflict drives the turnover of sex chromosomes. Although challenging, one approach would be to compare the autosome that has fused to the sex chromosome in both the ancestral population and the derived population to search for sexually antagonistic genes that might have driven the fusion. Finally, neo-sex chromosomes created by fusions provide a great system to study the early stages of sex chromosome evolution. Extensive genomic analyses have been conducted on the *Drosophila miranda* neo-sex chromosome created by a Y-autosome fusion; these studies have provided insights into the evolutionary forces that drive the degeneration of the Y chromosome (Bachtrog and Charlesworth 2002; Bachtrog 2003; Bachtrog 2004; Bachtrog 2006; Bachtrog et al. 2008; Bachtrog et al. 2009). Similar studies will be possible in fishes that have neo-sex chromosomes of a variety of ages, which will deepen our understanding of the process of sex chromosome evolution.

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