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DOI: 10.1534/genetics.108.098541

Linkage Analysis Reveals the Independent Origin of Poeciliid Sex Chromosomes and a Case of Atypical Sex Inheritance in the Guppy (*Poecilia reticulata*)

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Manuscript received November 10, 2008
Accepted for publication March 2, 2009

ABSTRACT

Among different teleost fish species, diverse sex-determining mechanisms exist, including environmental and genetic sex determination, yet chromosomal sex determination with male heterogamety (XY) prevails. Different pairs of autosomes have evolved as sex chromosomes among species in the same genus without evidence for a master sex-determining locus being identical. Models for evolution of Y chromosomes predict that male-advantageous genes become linked to a sex-determining locus and suppressed recombination ensures their co-inheritance. In the guppy, *Poecilia reticulata*, a set of genes responsible for adult male ornaments are linked to the sex-determining locus on the incipient Y chromosome. We have identified >60 sex-linked molecular markers to generate a detailed map for the sex linkage group of the guppy and compared it with the syntenic autosome 12 of medaka. We mapped the sex-determining locus to the distal end of the sex chromosome. We report a sex-biased distribution of recombination events in female and male meiosis on sex chromosomes. In one mapping cross, we observed sex ratio and male phenotype deviations and propose an atypical mode of genetic sex inheritance as its basis.

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LONG-standing models for evolution of sex chromosomes suggest that the process starts when a pair of autosomes acquires sex-determining genes in the vicinity of genes advantageous for only one sex (Muller 1914; Ohno 1967). This chromosome begins to differentiate from its homolog and suppressed recombination—due to accumulation of noncoding repetitive DNA sequences, pseudogenes, and transposable elements or chromosomal rearrangements—consolidates sex chromosome differentiation (reviewed in Charlesworth 2000; Charlesworth *et al.* 2005; Marshall Graves 2006).

During the gradual conversion of an autosome into a functional sex chromosome, it remains pseudoautosomal across most of its length for a major portion of its evolutionary lifetime. All the sex chromosomes identified in various fish species so far are considered to be at an early stage of differentiation, in contrast with mammals (Marshall Graves and Shetty 2001; Charlesworth 2004; Marshall Graves 2006). Hence, in typical fish sex chromosomes, there is only a small region with reduced crossing over, and homologous recombination is still possible throughout the larger pseudoautosomal portion. On the basis of studies of common ancestors and the viability of homozygous YY males, the age of teleost sex chromosomes has been estimated in a range of 2–30 million years, dependent on the species (Volff *et al.* 2007), compared to ~180 million years for the human Y chromosome.

The details of genetic sex determination in the major vertebrate groups vary considerably, especially in the teleost fishes, where a broad spectrum of sex-determining mechanisms can be found within the same order. Teleost species display unisexuality, environmental sex determination, and hermaphrodism in addition to heterogametic sex, which prevails in other classes (Volff 2005). Although male (XY) heterogametic sex is more frequent than female (ZW) heterogamety, both strategies have likely evolved several times independently in teleosts (Mank *et al.* 2006). In contrast to sex-determining loci (SDL), genes required for differentiation of the gonads are not necessarily located on the sex chromosomes, as deduced from hormone-induced sex reversal and from the presence of autosomal factors that can sometimes overrule the SDL on the male Y chromosome (Winge 1934; Winge and Ditlevsen 1938).

The only sex-determining gene identified so far in a teleost species is the DmrtY gene in the medaka, *Oryzias latipes*, on its Y chromosome (Matsuda *et al.* 2002; Nanda *et al.* 2002). The sex chromosomes of other species in this genus are derived from different ancestral

Supporting information is available online at http://www.genetics.org/cgi/content/full/genetics.108.098541/DC1.

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chromosomes (Takehana *et al.* 2007a; Tanaka *et al.* 2007). Similarly, the sex-determining loci of different salmonids (Phillips *et al.* 2005, 2007; Artieri *et al.* 2006) and of two species of stickleback (Gasterosteus) have apparently evolved from loci originating on distinct ancestral chromosomes (Woram *et al.* 2003; Charlesworth 2004; Peichel *et al.* 2004). In the three-spine stickleback *Gasterosteus aculeatus* (Peichel *et al.* 2004), the tiger pufferfish *Takifugu rubripes* (Kikuchi *et al.* 2007), the rainbow trout (Alfaqih *et al.* 2009), and the platyfish *Xiphophorus maculatus* (Volff and Schartl 2002), the SDL could be mapped to a specific linkage group that represents the differentiating Y chromosome. Fine mapping of these loci is impeded by suppression of meiotic recombination and by frequent occurrence of repetitive sequences in the gonosomal region of the Y chromosome (Nanda *et al.* 1993; Peichel *et al.* 2004).

Poeciliids are one of the best-studied groups of fishes with respect to sex chromosome evolution, due to the variable nature of sex determination in this family. In the genus Xiphophorus, there are three types of females, WY, WX, and XX, and two types of males, XY and YY. The nature of chromosomal sex determination in other genera is not very clear (Kallman 1973, 1984; Orzack *et al.* 1980; Volff and Schartl 2001; Schultheis *et al.* 2006).

Extensive genetic evidence supports a heterogametic XY sex determination in the guppy, *Poecilia reticulata* (Winge 1922a,b). Guppies are live bearers that display a pronounced sexual dimorphism for body size and for nuptial ornaments that are expressed exclusively in mature males. A substantial fraction of the ornamental genes is faithfully transmitted from father to son in every generation and may therefore represent male-advantageous genes linked to the SDL on Y in wild guppies (Winge 1927; Winge and Ditlevsen 1947; Haskins *et al.* 1970). Another set of ornamental genes can be maternally inherited, but their expression is largely limited to the sons. Similarly, the derived color patterns in ornamental guppies are linked to the SDL (Khoo 1999a,b,c). Some of the sex-linked traits were located relative to anonymous molecular markers, which were utilized to generate partial linkage maps for this species (Khoo *et al.* 2003; Watanabe *et al.* 2005; Shen *et al.* 2007).

As a first step toward identifying the genes and molecular mechanisms responsible for extreme phenotypic variations in most sex-linked traits of guppies, we have produced a detailed linkage map of the sex chromosomes, which reveals that the guppy sex-linkage group is syntenic with an autosome of medaka, which has an advanced draft of a genome sequence (Kasahara *et al.* 2007). Although male guppies are generally heterogametic, we describe a case of genetically atypical males and compare the patterns of meiotic recombination between males and females.

	1		2		3	4
F1 parent ♀	X^TX^T		X^TX^C		X^TX^C	X^TX^T
F1 parent ♂	X^TY^C or X^CY^T		X^TY^C or X^CY^T		X^TY^T	X^CY^C
F2 pool ♀	X^TX^T	X^TX^C	T>C	C>T	T>C	X^TX^C
F2 pool ♂	X^TY^C	X^TY^T	C>T	T>C	T>C	X^TY^C
	Sex linked				Inconclusive	

Figure 1.—BSA of SNP markers for sex linkage. Segregation of SNP markers among F_2 female and male pools from a single F_1 pair was analyzed for sex-linked pattern (see Figure S1). Among the four possible combinations of a biallelic SNP marker occurring in the F_1 parents, half were informative for sex linkage (examples 1 and 2), while the other half were not (examples 3 and 4). When the male F_1 parent is heterozygous and the female F_1 parent is homozygous (example 1), all F_2 individuals are informative. When both F_1 parents are heterozygous, 50% of their F_2 progeny are informative, resulting in signals of different strength for both alleles in pools of male and female F_2 (example 2). SNP markers are inconclusive when the male F_1 parent is homozygous (examples 3 and 4). Markers that appeared sex linked in F_2 pools were scrutinized in individual fish.

MATERIALS AND METHODS

Mapping crosses: Six mapping crosses between Quare and Cumaná guppy are described in a separate article by Tripathi *et al.* (2009). Briefly, F_1 individuals from each intercross were mated in single pairs to obtain F_2 progeny. We initiated a total of 26 intercrosses between guppies from Cumaná (Venezuela) and the Quare River (East Trinidad). Of 11 crosses between Quare females and Cumaná males (Q_Cu), 6 had >100 F_2 progeny and 2 had no offspring. Of 15 reciprocal crosses (Cu_Q), 2 had >100 F_2 progeny and 10 had no offspring. Compromised fertility in reciprocal intercrosses may indicate reproductive isolation (Russell and Magurran 2006) as a consequence of the Cumaná guppy being highly differentiated (Alexander and Breden 2004). Of the intercrosses with >100 F_2 progeny, we genotyped 5 (99, 150, 153, 157, 158) Quare ♀ × Cumaná ♂ and 1 (76) Cumaná ♀ × Quare ♂ (see supporting information, Table S2 for the number of individuals in each mapping cross). Anesthetized parents, F_1, and F_2 specimens were photographed as specified and then preserved in 95% ethanol at −20° (Tripathi *et al.* 2009).

Screening for sex linkage: The **b**ulk **s**egregant **a**nalysis (BSA) approach (Michelmore *et al.* 1991) was used for confirming sex linkage of all candidate genes. Briefly, the genomic DNA templates from 15 F_2 females and 15 F_2 males from a single F_1 pair of cross 76 (Cu_Q) were pooled. The primers were selected from the candidate coding genes either flanking an intron or from the 3′-UTR, and PCR was performed on each of the four templates. Single nucleotide polymorphisms (SNPs) were analyzed from these four templates (each F_1 parent and the F_2 female and male pools) for their segregation patterns to check for sex linkage of the selected candidates (Figure 1). On the basis of the parental genotypes, F_2 female and male pools were expected to show typical sex-linked segregation of the alleles (see Figure S1).

Synteny-based candidate gene selection: Homologs of confirmed sex-linked markers in the guppy were identified on genome scaffolds of other fishes, and adjacent regions were screened for the presence of coding genes (http://www.ensembl.org/index.html). Sequences of linked genes were then used to identify guppy homologs in an EST database

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TABLE 1

Detected recombination frequency between X and Y chromosomes in mapping crosses

Cross	Female	Male	X–Y recombinants (per total F_2)	Sex phenotype of recombinant F_2	X–Y recombination frequency (%)[a]
99	Qua6_203-4	Cumaná	3/192	3♀	1.56
150	Qua6_203-4	Cumaná	3/94	3♂	3.1
153	Qua6_203-4	Cumaná	1/269	1♀	0.37
157	Qua6_215-3	Cumaná	Proximal 7/854[b]	5 ♀ + 2 ♂	0.81
			Distal 1/854[b]	1♀	0.11
76	Cumaná	Qua6_215-3	Proximal 12/200[b]	8 ♀ + 4 ♂	6.0
			Distal 4/200[b]	3 ♀ + 1 ♂	2.0
16	Oropuche	Tranquille	1/52	1♂	1.9

[a] Highest detected recombination between X and Y averaged from all crosses is 2.29%, and the lowest is 1.49% for marker 0229 at the distal end.

[b] For cross 157 and for cross 76, numbers 7 and 1 and numbers 12 and 4 are the total number of recombinant F_2 individuals for markers at the proximal and distal ends of the sex chromosome, respectively.

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(http://guppy.weigelworld.org/weigeldatabases/) using BLASTN. Exon–intron boundaries of candidate genes were predicted on the basis of medaka, fugu, and tetraodon sequences, and intron-flanking primers were designed from each candidate gene. All markers found to be sex linked by BSA were subsequently confirmed by sequencing of PCR products from DNA templates from individual fish (Table S1a).

Genomic walking: A genomic BAC library of eightfold coverage and an average insert size of 160 kb were prepared from pooled Cumaná males (constructed by Bio S&T, Montreal). Filters with spotted BAC clones were prehybridized in 5× SSC, 0.02% (w/v) SDS, 0.1% (w/v) *N*-lauroylsarcosine, and 1% blocking solution for 4 hr at 60°. Probes representing known sex-chromosome-linked sequences were prepared with a PCR DIG-probe synthesis kit (Roche), and filter hybridization was carried out overnight at 60°. Filters were washed three times each in 0.1% SDS, 2× SSC (30 min at 60°); 0.1% SDS, 2× SSC (20 min at 60°); and 0.1% SDS, 0.5× SSC (15 min at 60°). Membranes were treated with blocking solution, followed by 1:10,000 (v/v) diluted anti-DIG antibodies (Roche). Detection was performed using CSPD chemiluminescent substrate (Roche). Blots were exposed to Lumi-film chemiluminescent detection film (Roche) for 20 min to several hours. Clones identified by significant hybridization signals were streaked on chloramphenicol plates, and single colonies were picked for inoculation of overnight cultures for DNA preparation. BAC DNA was isolated using a Qiagen large-construct kit and tested by PCR for the presence of the genomic fragment used for filter hybridization. Each BAC was sequenced at both ends with Big Dye Terminator chemistry using the standard pIndigoBAC-5 vector-specific sequencing primers forward 5′-GGA TGT GCT GCA AGG CGA TTA AGT TGG and reverse 5′-CTC GTA TGT TGT GTG GAA TTG TGA GC on an Applied Biosystems 3730xl DNA Analyzer. The obtained sequences, which mostly represented noncoding DNA, were used to design primers for PCR on genomic templates. The resulting novel SNP markers were tested by BSA performed with pooled male and female templates as described above to verify sex linkage. PCR on DNA from all BACs isolated during a genomic walk was used to determine the extent of overlap between these BACs.

Mapping of *Sex*: For mapping *Sex*, >2000 offspring from six mapping crosses between the Quare and Cumaná populations were used, as specified in Table S2. Phenotypic sex was scored by presence of adult male ornaments and a differentiated gonopodium. Genotypes for 790 SNP markers of each individual were analyzed with Joinmap4 (Van Ooijen 2006) to determine the location of *Sex* in the genomewide linkage map. Recombination frequencies of the markers on the sex linkage group were estimated on the basis of X- and Y-linked alleles segregating in each mapping cross, with phenotypic sex as the reference (Table 1). The exact sex chromosome composition for each recombinant individual was predicted from the genotypes of the sex-linked markers and correlated with phenotypic sex.

Synteny determination with medaka: BLASTN and BLASTX were performed for all sex-linked markers from the guppy against the medaka genome. The exact position for each marker on medaka chromosome 12 was converted on a scale of 1–30 for comparison of the relative orders and gaps between markers with the guppy LG 12 map. The individual maps were plotted using MapChart (Voorrips 2002).

RESULTS

Identification of sex-linked markers: BSA (see materials and methods) allows identification of sex-linked genes whose male and female alleles may be distinguished by SNPs. As shown in Figure 1, a SNP is informative for sex linkage when a male F_1 is heterozygous for this marker. For this strategy, the F_1 parents and the pools of F_2 male and female genomic DNA were used as a template for PCR with the candidate primers (described in materials and methods). The sequence traces of the PCR products were inspected for segregation of SNPs identified in the grandparents (Figure 1 and Figure S1).

One of 58 SNP markers tested identified the gene encoding cyclin G2 as sex linked. Additional markers were developed by analyzing the sex linkage of guppy homologs of genes that were located on the same genomic scaffolds as cyclin G2 in medaka, fugu, or tetraodon (see materials and methods and Table S1a and Table S1b). Using this synteny-based candidate selection approach, 20 additional sex-linked markers could be identified in the guppy. As a complementary strategy, we pursued genomic walking with BAC clones.

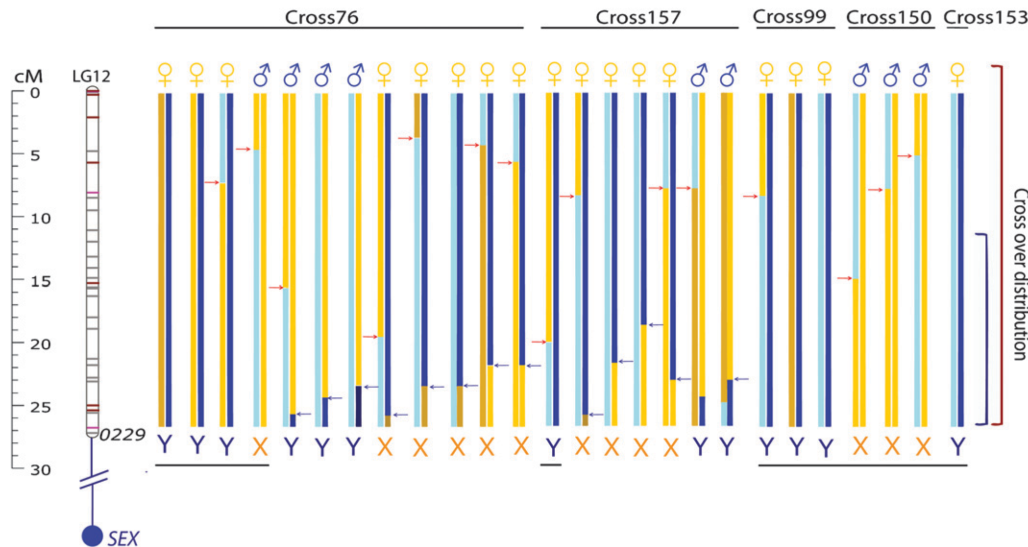

Figure 2.—Recombinant F_2 individuals for X and Y chromosomes in five mapping crosses. Each bar represents the recombined sex chromosomes of an F_2 individual. The X chromosome inherited from the F_1 female parent is represented by the left half of each bar, while the right half of each bar represents the sex chromosome from the F_1 male parent. The light and dark blue colors show the paternal strain (grandfather) derived X and Y chromosomes, respectively. The X chromosomes from the maternal strain (grandmother) are in yellow in each recombinant. The phenotypic sex of each recombinant is shown by symbols at the top. The presence of either an XX or an XY genotype for mapped markers at the distal end of every recombinant is indicated at the bottom as X (yellow) or Y (blue). The putative *Sex* locus is predicted to be near the distal end of the chromosome, downstream of the last mapped marker (0229). Horizontal black lines mark recombinants, for which the sex chromosome genotype at the distal end disagrees with phenotypic sex. A single crossover event between marker 0229 and the *Sex* locus could explain their phenotypic sex. These recombinants were used to estimate the total recombination frequency at this end of the linkage group (see Table 1). The positions of the crossover events along the sex chromosome in female and male meiosis are shown by red and blue arrows, respectively. The distribution of the crossover events in female and male meiosis is represented by the vertical red and blue brackets on the right.

Additional markers came from the development of a whole-genome linkage map for the guppy, described in Tripathi *et al.* (2009). Table S1a and Table S1b show the complete list of >60 sex-linked markers generated with the three approaches.

Recombination frequency on the sex linkage group and mapping of *Sex*: Most sex chromosome markers were homozygous in the parental strains and were therefore informative in only 50% of the F_2 progeny. The markers that could distinguish between X- and Y-linked alleles in a cross were used to estimate recombination frequencies between the two chromosomes (Figure S1). F_2 progeny from five mapping crosses between Cumaná and Quare populations were used to estimate the genetic position of the dominantly acting sex-determining locus, *Sex*.

Utilizing the information from X- and Y-linked alleles, the position of crossover events was noted for every recombinant sex chromosome. Table 1 shows the recombination frequencies detected from all informative sex chromosome markers in different mapping crosses. Cross 16 is a cross between two additional populations (Oropuche ♀ × Tranquille ♂), analyzed for a subset of sex-linked markers. Across all crosses, the highest recombination frequency for any marker with *Sex* was 2.3% and the closest to *Sex* was marker 0229, with 1.5% recombination frequency. Recombination between X chromosomes in XX females had identified this marker as the last genetic marker mapped on the sex chromosome, placing *Sex* distal to marker 0229.

Among five mapping crosses with >1300 F_2 individuals (Table S2), we found 26 F_2 with recombinant sex chromosomes (Figure 2). For 14 of these individuals, the genotype at marker 0229 agreed with the phenotypic sex (XX female, XY male, Figure 2). A single crossover event between the most distal marker (0229) and the *Sex* locus in their F_1 father can explain the phenotypic sex of the other 12 individuals. We therefore infer the occurrence of such crossover events, although additional molecular markers are required to confirm this at the DNA level.

Nonrandom distribution of crossovers between X and Y chromosomes: In cross 157 (Q_Cu), <1% (7/854) of F_2 animals had a recombinant sex chromosome, while, in the reciprocal cross 76 (Cu_Q), 6% (12/200) of F_2 individuals were found to have a recombinant sex chromosome (Table 1). Recombination between the distal-most marker (0229) and *Sex* was detected in only one of the cross 157 individuals, but in four of the cross 76 progeny. The resulting estimates of the recombination frequency between marker 0229 and the *Sex* locus are therefore 0.1% for cross 157 and 2.0% for cross 76 (Table 1).

Crosses 99, 150, and 153 (all Q_Cu) produced 1.6, 3.3, and 0.4% recombinants, respectively. These were phenotypic females in cross 99 and cross 153, and males in cross 150. The recombination events in all of these individuals are predicted to have occurred between marker 0229 and the *Sex* locus.

By analyzing the distribution of crossover events along the sex chromosomes, we found a stark difference between female and male meiosis. Recombina-

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tion events in the F_1 female meiosis were evenly distributed along the length of the X chromosome. In contrast, crossovers in F_1 male meiosis clustered toward the distal end of the chromosome (blue arrows in Figure 2). This observation was consistent with the difference between the reciprocal crosses 76 and 157, which had produced 12 and 7 recombinant individuals, respectively.

Synteny between the guppy sex chromosome and medaka chromosome 12: We identified homologs of the guppy sex chromosome genes in the medaka *O. latipes* using BLASTN. All but three of these are located on medaka chromosome 12, with the order along the chromosome largely syntenic with the order of the guppy sex chromosome loci (Figure 3). Medaka homologs of several additional sequences identified by genomic walking in a guppy BAC library are also located on chromosome 12. Many sequences identified by genomic walking toward the distal end of the guppy sex chromosome were rich in repeat sequences, making it impossible to place these on the genetic map.

Comparative mapping has demonstrated that chromosome 12 of *O. luzonensis*, in the same genus as *O. latipes*, contains its SDL (Tanaka *et al.* 2007) at a position corresponding to between markers 0090 and 0210 on the guppy sex chromosome. Considering that *Sex* is located at the extreme distal end of LG 12 in the guppy, we think that it is unlikely that the sex determination locus is conserved between the guppy and *O. luzonensis.*

Atypical sex inheritance: We found an atypical segregation ratio of Y-linked male traits in cross 158 (Q_Cu) with more males than females (Figure 4, A and B), in contrast to the expected 1:1 sex ratio that was normally observed in all other mapping crosses.

The Cumaná male in cross 158 had three Y-linked traits (YLT) typical for this population, including a central blue white spot (CBWS) ventral to the dorsal fin, a dorsal fin orange and black (DFOB), and a posterior ventral black stripe (PVBS) on the caudal peduncle. In addition, some color traits are X and Y linked (X-YLT), the most important being the hind-fin black spot (HFBS) and the hind-fin lower orange (HFLO), in Figure 4A, left fish.

The F_1 generation from cross 158 included 21 females and 41 males (♀:♂ = 1:2). Among the F_1 males, two strikingly different phenotypes could be distinguished by either the presence or the absence of the three Y-linked traits, with 22 males lacking and 19 males having them (ratio 1:1). The F_2 sons of F_1 males lacking the Y-linked traits (pairs 11, 14, and 16) also did not express these traits. Among the normal-appearing F_1 males, some faithfully passed on the YLT to the F_2 generation (pairs 6, 7, and 15) while other normal-looking F_1 males from three pairs (5, 9, and 10) had half their F_2 male offspring without the YLT. In addition, F_2 sex ratios were very skewed (Figure 4B).

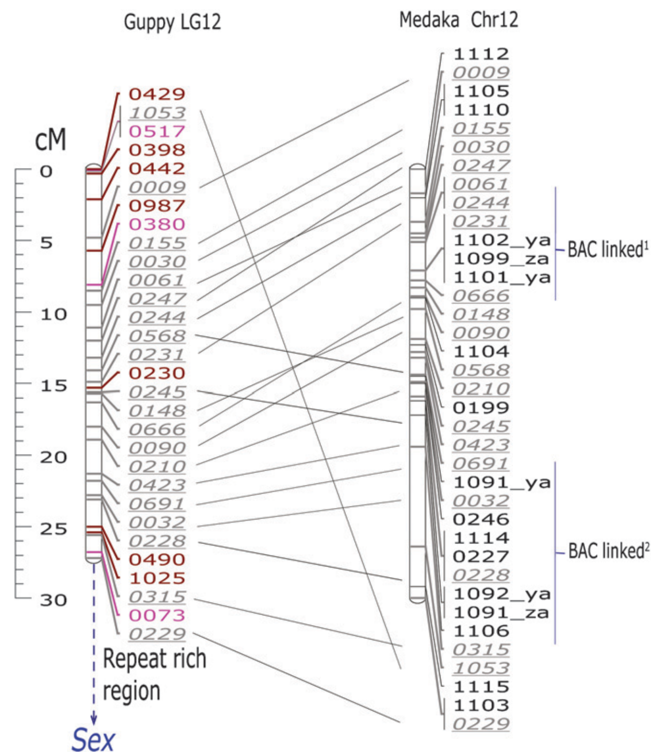

Figure 3.—Synteny relationships between medaka chromosome 12 and guppy LG 12. Markers common between the two maps, with known positions in both, are underlined in gray. Markers in pink on guppy LG 12 map to different chromosomes of medaka (0380 to chromosome 15, 0517 to chromosome 03, and 0073 to chromosome 14 of medaka). Markers in brown on guppy LG 12 do not have a significant BLAST hit on the medaka genome. Markers in black on medaka chromosome 12 are sex linked in the guppy, and their approximate positions are predicted from the synteny of linked markers, but not shown on the guppy map. BAC linked[1,2] (thin blue lines on the right) include markers derived from BAC end sequences during genomic walking. Additional BAC-linked markers from the same region, with no homolog identified on medaka chromosome 12, are listed in Table S1. Several BAC clones resulting from genomic walk toward the distal end of guppy LG 12 were rich in repeat sequence that interfered with reliable detection of sex linkage. The putative location of the master SDL is at the distal end of the guppy LG 12.

All the F_1 and F_2 progeny of this cross were genotyped with a set of SNP markers for genomewide linkage analysis (Tripathi *et al.* 2009). In a few cases, it was possible to differentiate between all four sex chromosomes involved in the cross from the Quare female ($X^{Q1}X^{Q2}$) and from the Cumaná male ($X^{C}Y^{C}$) using the marker genotypes (see Table S3) and to follow their segregation in the subsequent generation. Correlating the presence of the different chromosomes and the abnormal segregation of sex and Y-linked traits revealed that the X^{Q2} chromosome was responsible for both. This was evident from the observation that all F_1 females of this cross had the $X^{Q1}X^{C}$ genotype, while F_1 males not only were as expected—$X^{Q1}Y^{C}$ and $X^{Q2}Y^{C}$—but also were $X^{Q2}X^{C}$. These three genotypes furthermore were clearly

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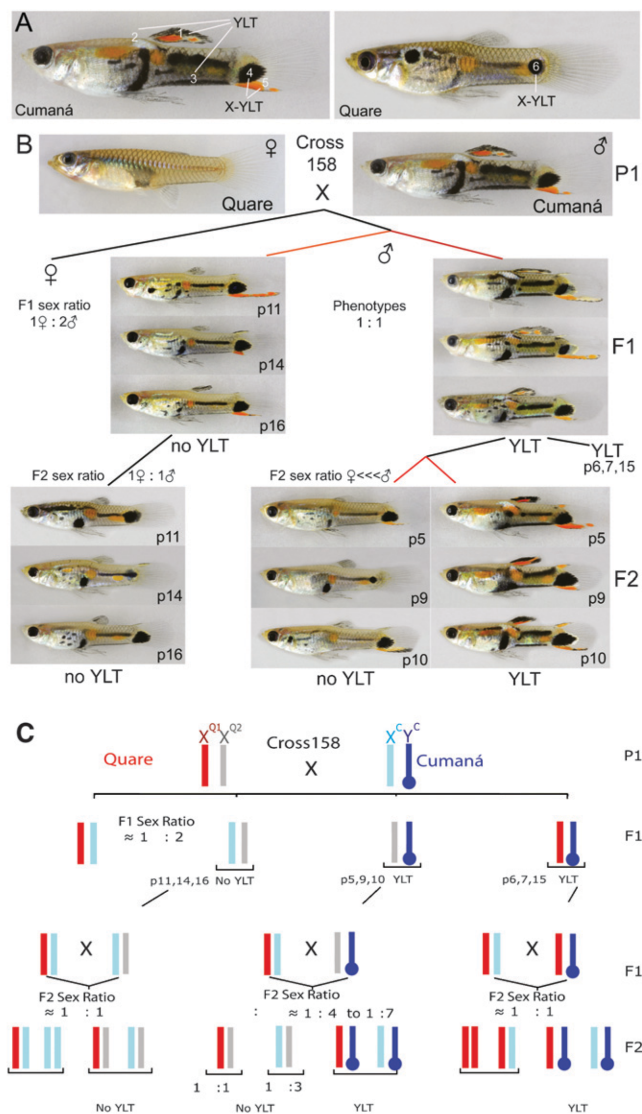

Figure 4.—Summary of cross 158 atypical sex determination. (A) The three YLTs segregating in cross 158 are marked on the left image of the Cumaná male parent of this cross: (1) dorsal fin orange and black (DFOB), (2) central blue white spot (CBWS), and (3) posterior ventral black stripe (PVBS). The X- or Y-linked traits (X-YLT) of the Cumaná population are(4) the hind-fin black spot (HFBS) and (5) the hind-fin lower orange (HFLO). The right fish shows the Quare X- or Y-linked trait (6), a black spot on the caudal peduncle, which shows a mutually exclusive expression with respect to the HFBS (4) from Cumaná. (B) Representative males showing segregation of the phenotypes of three YLTs and the sex ratios in F_1 and F_2 generations. Three kinds of F_1 males with genotypes as explained in C were found. F_1 males without YLTs gave rise to all F_2 males with no YLT (pairs 11, 14, 16). F_1 males of genotype $X^{Q1}Y^{C}$-sired F_2 males with YLTs (pairs 6, 7, 15). F_1 males of genotype $X^{Q2}Y^{C}$ had YLTs and gave rise to predominantly male F_2 of which 50% had YLTs (pairs 5, 9, 10; shown with red lines). See Table S2 for the number of individuals in each category and sex ratios. (C) The segregation of the sex chromosomes in cross 158, which correspond to each of the phenotypes displayed in B. The four sex chromosomes segregating in this cross were identified by specific SNP markers in each (Table S3) and are shown in four different colors. All males with Y^{C} had three YLTs whereas males whose sex was conveyed by X^{Q2} had no YLT. The recombinant sex chromosomes for any of the genotypes are not shown in the figure.

correlated with the three classes of the F_1 males described above. All $X^{Q1}Y^{C}$ F_1 males and their F_2 progeny showed normal Y-linked traits and normal sex ratios. In contrast, F_2 progeny of $X^{Q2}X^{C}$ males displayed a 1:1 sex ratio but the males lacked YLT. Whereas $X^{Q2}Y^{C}$ males expressed normal YLT, half of their F_2 male progeny lacked them. Moreover, there was an up to sevenfold excess of sons among the F_2 progeny.

All these results can be explained by postulating that the X^{Q2} chromosome from the Quare female parent carries a factor that supports the development of fully fertile males in the absence of a Y chromosome (Figure 4C). An important clue was provided by the presence of two sex-chromosome-linked phenotypic traits, the HFBS on Cumaná and the posterior black spot on the caudal peduncle of Quare (Figure 4A), which is X and Y linked in Quare individuals. The HFBS from Cumaná is dominant over the Quare black spot and is always expressed if either an X^{C} or Y^{C} allele for this trait is present.

Analysis of the sex-chromosome-recombinant F_2 individuals from cross 158 indicated that the markers at the distal end of the sex chromosome were correlated with the phenotypic sex of the XX individuals. The unusual X^{Q2} locus responsible for male phenotypic sex is therefore predicted to be toward the distal end of the sex chromosome.

We compared the distribution of crossover events in the F_1 females ($X^{C}X^{Q1}$) with atypical F_1 males ($X^{C}X^{Q2}$) from cross 158. For this purpose, we analyzed a total of 87 F_2 offspring from three F_1 pairs (11, 14, and 16) of cross 158, which had $X^{C}X^{Q2}$ males as their F_1 parent. Interestingly, recombination frequency on the sex chromosome was lower in the F_1 males compared to F_1 females of this cross, even though the genotype of males is XX and not XY (supporting information, Figure S2; supporting information, Table S4b. In addition, the distribution of the crossover events along the length of the chromosome displayed a pattern similar to that observed from meiosis in normal XY males of other crosses (Figure 2). The majority of crossovers in male meiosis occurred toward the distal end of chromosome, while no such bias was observed in female meiosis.

The different recombination frequencies observed in female and male meiosis could be caused by sequence differences between the X and Y chromosomes. However, the clustering of crossovers does not correlate with the expected gradient of recombination suppression in the vicinity of the differentiated region of the sex chromosomes. In addition, the $X^{Q2}X^{C}$ males in cross 158, like normal XY males, show a similar pattern of reduced recombination as well as a biased distribution of crossover events along the sex chromosome (Figure

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S2; Table S4a and Table S4b). These results indicate that recombination is controlled by phenotypic sex.

DISCUSSION

Mapping of the Y-specific region of the sex chromosome: The Y-specific "gonosomal" region containing the male SDL and a number of male-specific color genes has previously been postulated to be at one end of the guppy sex chromosomes by Winge (1927). Later, the Y-specific segment has been clearly detected at a terminal location in mitotic and meiotic spreads by chromosome *in situ* hybridization, comparative genomic hybridization, and synaptonemal complex analysis (Nanda *et al.* 1992; Traut and Winking 2001). Consistent with this, predictions from our results suggest a distal location of the Y-specific segment and the *Sex* locus. An extensive region of sequence homology, as well as several polymorphisms detected in the pseudoautosomal regions of X and Y chromosomes, indicate that the molecular rearrangements differentiating the sex chromosomes is still ongoing. In support of this prediction, no difference was found in allele distributions between X and Y when ~250 specimens from 42 wild guppy populations were genotyped, using all mapped sex-linked markers (data not shown).

Population-specific divergence of sex chromosomes: It is striking that cross 76, which is the only genotyped cross between a Cumaná mother and a Quare father, shows a much higher number of recombination events between X and Y compared to the five reciprocal crosses. Although this difference could be a random variation, it could also indicate that the sex chromosomes are diverged to different degrees between these two populations. If the Y is more differentiated from the X in Cumaná compared to the Quare population, a higher rate of recombination is expected between the X and Y chromosomes of Quare (as in cross 76) relative to the recombination rate between the X and Y chromosomes of Cumaná (reciprocal crosses). Since the order of mapped sex-linked markers showed no differences between cross 76 and the reciprocal crosses, any potentially significant differences between the sex chromosomes of the Cumaná and Quare populations should be limited to the distal region of the chromosome not yet covered by our markers.

Synteny of the guppy sex chromosome with medaka chromosome 12: The order of markers between the sex linkage group of the guppy and homologous chromosome 12 of *O. latipes* (Figure 3) are mostly conserved. One significant translocation is revealed by the position of marker 1053 at the proximal end, which is present toward the distal end of medaka chromosome 12. The gap between marker 1053 and marker 0229 is only 2.93 Mb on medaka chromosome 12, whereas they map to two ends of guppy chromosome 12. A comparison with other fish genomes revealed these markers to be linked within a 1.9-Mb segment on stickleback group XVI and to occur within 600 kb on homologous scaffold 106 in Fugu. These observations suggest that the chromosomal segments mapping to either end of guppy chromosome 12 were linked closely in the ancestral populations of these fish species.

Linkage analysis of the sex chromosomes of guppy and *O. luzonensis* indicated that despite a shared synteny with *O. latipes* chromosome 12, these two species have different sex-determining loci. This is not surprising, considering the general lack of conservation of the master sex-determining gene among teleost species (Mank *et al.* 2006; Volff *et al.* 2007). At least four different chromosomes gave rise to the SDL in different species of Oryzias, suggesting that several master sex-determining genes may have evolved even in closely related species (Takehana *et al.* 2007a,b; Tanaka *et al.* 2007). The *O. latipes* Dmrt1bY containing the Y-specific segment is only 250 kb in length, while the rest of the Y is completely homologous with the X chromosome (Kondo *et al.* 2006), suggesting that a small segment of DNA, having at least one important functional gene for male sex determination, is sufficient to serve as a focal point for sex chromosome divergence.

Sex determination in the guppy can deviate from the XY paradigm: Winge (1930, 1934) has shown that it was possible to obtain XX males in the guppy, and he also proposed that multiple antagonistic autosomal factors may contribute to the ultimate outcome of genetic sex determination. The male sex differentiation linked to one specific X chromosome was not universally observed in the Quare population but appears to be specific to the female Quare parent of cross 158. From the available markers, we cannot deduce whether the putative sex-determining gene near the distal end of the X^{Q2} chromosome is derived from the normal Y-linked male sex determinator itself or by recombination from another cryptic sex chromosome occurring in the Quare population. Alternatively, this X^{Q2}-linked gene might encode a downstream component of the male sex-determining pathway, and a mutation or recombination might have altered its regulation. In either of these scenarios, a gene now found on the X^{Q2} may function as a male sex-determining gene. A distinction between these possibilities will require denser marker coverage and sequence information from the distal end of this chromosome. The X^{Q2} was found to behave as a stable male-determining chromosome in the Quare genomic background throughout more than five generations of backcrosses derived from cross 158 F_1 males (data not shown). Therefore, different combinations and ratios of additional autosomal factors might direct the regulation of the sex differentiation toward the male or female pathway.

Sex-specific meiotic recombination on chromosome 12: The crossover events found in male meiosis were predominantly restricted to a limited boundary region

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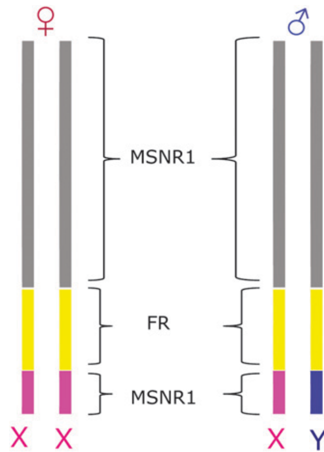

Figure 5.—Model for the organization and evolution of guppy sex chromosomes. The three regions of the sex chromosome differentiated on the basis of recombination in male meiosis: male-specific nonrecombining 1 (MSNR1), freely recombining (FR), and male-specific nonrecombining 2 (MSNR2). The phenotypic sex of the individuals depends on the genotype of the distal (MSNR2) segment only, XX female and XY male, irrespective of the composition of the rest of the chromosome.

between the pseudoautosomal and the putative sex-differentiated segment of the sex chromosome (Figure 2). Differences in meiotic recombination frequencies depending on phenotypic sex have been described in medaka fish (Yamamoto 1961; Matsuda *et al.* 1999; Kondo *et al.* 2001), where the recombination frequencies on the sex chromosomes of normal and sex-reversed individuals showed a clear correlation with their phenotypic sex. Irrespective of their sex chromosomal genotypes, phenotypic males displayed a lower recombination frequency on the sex chromosomes than phenotypic females did. Sex-specific variation in recombination frequency and distribution is observed in many, including mammals, but no clear molecular basis has yet been found to explain these differences (Lynn *et al.* 2005).

Model for the organization and evolution of guppy sex chromosomes: On the basis of our observations on the nonrandom distribution of meiotic recombination on guppy sex chromosomes and our mapping results, we propose a model for the organization of the guppy sex chromosomes. According to this model (Figure 5), the guppy sex chromosome is constituted by three distinct regions, namely male-specific nonrecombining 1 (MSNR1), freely recombining (FR), and male-specific nonrecombining 2 (MSNR2). The set of mapped markers in this study covers the MSNR1 region and a part of the FR region. From the distribution of all the mapped markers on the sex chromosome and the preliminary results of chromosome *in situ* hybridization (I. Nanda, N. Tripathi, C. Dreyer and M. Schantl, unpublished results), we predict these regions to represent ~60% (MSNR1), 10–20% (FR), and 20–30% (MSNR2) of the total length of the sex chromosome (Figure 5).

These three regions can be differentiated only on the basis of the recombination suppression in male meiosis, as all three regions recombine freely between the X chromosomes during female meiosis. MSNR1 shows suppressed recombination during meiosis in phenotypic males, in contrast to FR, which either has escaped sex-specific recombination suppression or overrules it. MSNR2 is the region that represents the differentiated part of the sex chromosome, including the gonosomal region of Y with the sex-determining and Y-linked color loci (Figure 5). The sequence composition and length of this segment might be variable between guppy populations.

While sex-dependent recombination suppression of the distal gonosomal region of the Y chromosome can be explained by sequence divergence, *e.g.*, by inversions or insertions, the mechanism by which meiotic recombination also becomes restricted in the pseudoautosomal region (MSNR1) remains unclear. Positioning of a recombining region at the boundary (FR) of the diverged segment of the sex chromosome (MSNR2) could be under favorable. Meiotic recombination of the chromosome at the interphase between the pseudoautosomal and gonosomal regions offers a mechanism that protects the major portion of the sex chromosome against accumulation of deleterious mutations while maintaining the diverged region of the chromosome, which links male-advantageous traits to a SDL. Recombination in this region may also provide a mechanism for maintenance of enhanced variation among X- and Y-linked male color traits. The characteristic male color variation is regarded as important for both natural and sexual selection in guppies. Consequently, enhanced recombination of the FR could be advantageous for at least two reasons: purging of deleterious mutations and enhanced natural variation. From an evolutionary perspective, this model allows for an easy translocation of a small diverged segment of a sex chromosome to an autosome, potentially initiating the evolution of novel sex chromosomes.

We thank Christa Lanz for help with sequencing, Eva-Maria Willing for statistical analysis, Aleks Basara for help with illustrations, Indrajit Nanda for discussion, Manfred Schartl for comments on the manuscript, and the reviewers for constructive criticism. This work was supported by a Gottfried Wilhelm Leibniz Award of the Deutsche Forschungsgemeinschaft and by the Max Planck Society.

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Linkage Analysis Reveals the Independent Origin of Poeciliid Sex Chromosomes and a Case of Atypical Sex Inheritance in the Guppy (*Poecilia reticulata*)

Namita Tripathi, Margarete Hoffmann, Detlef Weigel and Christine Dreyer

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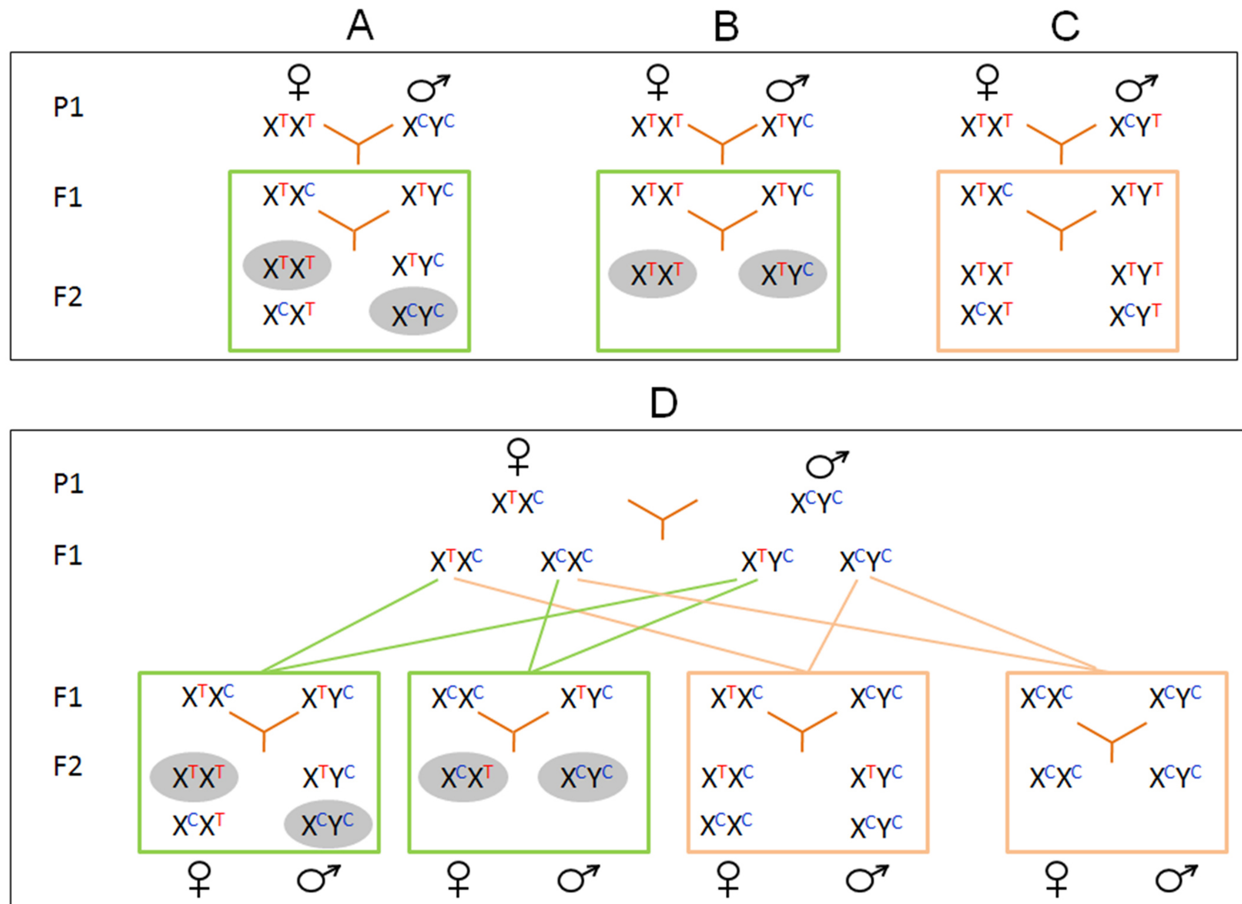

FIGURE S1.—Segregation pattern of different sex linked SNP markers in F2 population. (A) ♀ and ♂ Grandparents homozygous for opposite sex linked alleles. All ♀ and ♂ F1 offspring are heterozygous for such a marker and only 50% of the total F2 individuals (25% ♀ and 25% ♂) have distinctly sex specific genotypes (shaded grey). (B) ♀ Grandparent is homozygous and ♂ grandparent has a Y- linked SNP. All ♀ F1 offspring are homozygous and ♂ F1 offspring are heterozygous for such a marker and 100% of the F2 individuals are informative for sex linkage (shaded grey). (C) ♀ Grandparent is homozygous and ♂ grandparent has an X- linked SNP. All ♀ F1 offspring are heterozygous and ♂ F1 offspring are homozygous for such a marker and the F2 individuals are not informative for sex linkage. (D) ♀ Grandparent is heterozygous and ♂ grandparent is homozygous for the sex linked SNP marker. 50% F1 offspring are heterozygous and 50% are homozygous (♀ and ♂) for such a marker. Depending on the F1 ♀ parent genotype, either 50% or 100% of the total F2 individuals can be informative when the F1 ♂ parent has heterozygous alleles shaded grey). When the F1 ♂ parent is homozygous, the F2 individuals are not informative for sex linkage.

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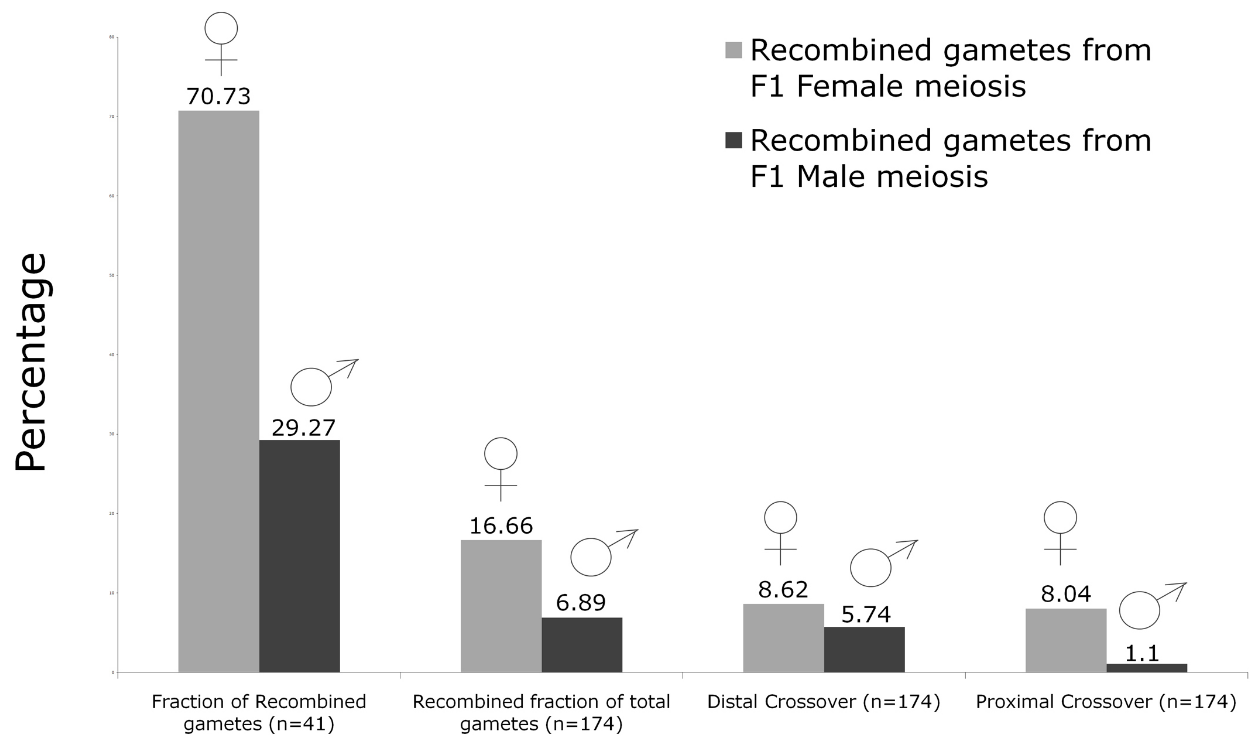

FIGURE S2.—Analysis of sex chromosome recombination frequency and distribution in F1 meiosis from cross158-Pairs11,14 and 16. In 87 F2 progeny from F1 parents with female X^CX^{Q1} and male X^CX^{Q2} genotypes 41 of 174 sex chromosomes were found to be recombined. Distal refers to 10-20% of the mapped distal end of the sex chromosome (corressponding to FR segment in Figure 6) and proximal refers to approximately 60% of proximal region of the sex linkage group (synonymous to the MSNR1 segment from Figure 6); n= Number of gametes. (See Table S4)

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TABLE S1a

Annotation of sex-linked genetic markers of *Poecilia reticulata*

Marker	Origin	Guppy clone	Genbank Accession	Annotation of possible function (BLAST results)
0009	EST	Tra_Embryo_15_D19	ES375672	Rab interacting lysosomal protein-like 1
0030	EST	Qua_Embryo_U2_N18	ES382008	**Fructose-1,6-bisphosphatase 1 [Danio rerio]**
0032	EST	Tra_Embryo_8_L19	ES386074	**Steroid-sensitive protein 1 [Danio rerio]**
0061	EST	Qua_Embryo_B2_E02	ES379217	**Ectodermal-neural cortex 1 ENC1**
0073	EST	Qua_Embryo_C1_D11	ES379375	**Replication factor C (activator 1) 3** [Danio rerio], **CAG00421**, [Tetraodon nigroviridis] (Medaka Chromosome 14
0090	EST	Blu_Brain_U2-3_E08	ES372993	N-Shc (1E-50)
0148	EST	Blu_Testis_6_H06	ES380905	**Calmodulin regulated spectrin-associated protein 1**
0155	EST	Oro_Skin_3_K13	ES385036	Splicing factor, arginine/serine-rich 12 (1.2E-31)
0199	EST	Blu_Brain_L2_H11	ES372663	**Thyroid hormone receptor-associated protein 2**
0210	EST	Blu_Testis_6_K19	ES380974	**Heat shock 70kDa protein 5 (glucose-regulated protein 78kDa)**
0229	EST	Tra_Embryo_10_F17	ES374479	**Cyclin I**
0230	BAC	24-K15	FH890169	NS
0244	EST	Oro_Retina_5_C12	ES373878	**TGF beta-inducible nuclear protein 1 (CDK105)**
0245	EST	Tra_Embryo_15_F16	ES375709	**Catenin ARVCF [Danio rerio]; DW618591**
0246	EST	Tra_Embryo_2-1_F11	ES371278	**NADH dehydrogenase ubiquinone 1 alpha subcomplex, 8, 19kDa**
0247	EST	Tra_Embryo_1-4_G05	ES371208	**Transcription factor BTF3a**
0315	BAC	01-4_H01	FH888831	NS
0380	BAC	03-F10	FH889280	(Medaka Chromosome 15) The start of this sequence is located in Contig scaffold91_contig45820
0398	BAC	03-J09	FH889369	NS
0423	BAC	03-P19	FH889502	Fundulus heteroclitus cDNA similar to PDZ and LIM domain protein (5e-42)
0429	BAC	04-G05	FH889541	Guppy EST Tra_Liver_5_C16 (ES377721.1; 2e-92)
0442	BAC	04-A18	FH889605	Guppy EST Tra_Liver_5_N20; REX2, microsatellite Msd018
0490	BAC	04-B08	FH889775	NS

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0517	BAC	05-C08	FH889941	(Medaka Chromosome 03) Contig scaffold312_contig85780,3 coding genes near by: ENSORLG00000015448, ENSORLG00000015460, ENSORLG00000015469
0568	BAC	32-L01	FH890372	RHO GTPASE ACTIVATING RHO TYPE GTPASE ACTIVATING
0603	BAC	33-E18	FH890561	C07125rat cDNA (2e-15)
0666	BAC	34-L18	FH891090	BJ731761 MF015DA cDNA Oryzias latipes cDNA (4e-46)
0691	BAC	01-G08	FH891254	Medaka ENSORLG00000009417; Vitamin K-dependent gamma-glutamyl carboxylase (4e-42)
0987	BAC	33-D15	FH893254	NS
1025	BAC	34-K02	FH893550	NS
1053	EST	Tra_Liver_7-4_H05	ES377437	JanusA / ocnus (8.00E-21); phosphohistidine phosphatase 1 (3e-51)
1075	Homology cloning		FJ200253	**aim1 slc45a2 membrane-associated transporter protein B**
1079	Homology cloning		FJ236234	**phosphodiesterase 6A**
1103	EST	Tra_Embryo_3-3_G01	ES371695	**Cyclin G2**
1104	Consensus	Medaka ZBTB7C§	ENSORLG00000005778	ZINC FINGER AND BTB DOMAIN CONTAINING Protein
1105	Consensus	Medaka LIN54§	ENSORLG00000001906	TESMIN METALLOTHIONEIN 5
1106	EST	Qua_Embryo_02_D01	ES383177	BX901878.6 GI:60098192 on Zebrafish linkage group 21
1107	EST	Tra_Liver_4_F16	ES377165	Unnamed protein product [Tetraodon nigroviridis] gi|47210133|emb|CAF95582.1|
1108	EST	Qua_Embryo_U5_M02	ES378762	Ab012309 Cyprinus carpio mRNA for **allograft inflammatory factor-1**
1109	EST	Blu_Testis_8_E24	ES380544	**TIA1 cytotoxic granule-associated RNA binding protein** [Danio rerio]
1110	EST	Blu_Testis_8_P16	ES380724	**COP9 signalosome subunit 4**
1111	EST	Blu_Testis_6_H23	ES380918	**Restin** (Reed-Steinberg cell-expressed intermediate filament-associated protein)
1112	EST	Blu_Testis_4_A23	ES379967	**Zinc finger FYVE domain-containing endosome-associated FYVE-domain protein**
1113	EST	Qua_Embryo_01_C04	ES383103	**beta A4-crystallin**
1114	EST	Blu_Testis_8_G09	ES380567	**Putative alpha-mannosidase C1orf22**
1115	EST	Oro_Skin_4_O13	ES384849	Thioredoxin-like 1
0228; 1080_ya	BAC	34-P12	FH893402; FH890782	NS
0231; 1081_ya	BAC	08-M18	FH892752; FH890160	NS

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1082_za; 1082_ya	BAC	24-K15	FH892762; FH890169	NS
1083_za; 1083_ya	BAC	18-N08	FH892760; FH890167	NS
1084_za; 1084_ya	BAC	01-H04	FH891124; FH888484	NS
1085_za; 1085_ya	BAC	02-P16	FH891473; FH888843	NS
1086_za; 1086_ya	BAC	10-H16	FH892753; FH890161	NS
1087_za; 1087_ya	BAC	17-F23	FH892756; FH890164	NS
1088_za; 1088_ya	BAC	02-J05	FH891472; FH888842	NS
1089_za; 1089_ya	BAC	18-C03	FH892759; FH890166	NS
1090_za; 1090_ya	BAC	20-M10	FH892761; FH890168	NS
1091_za; 1091_ya	BAC	40-A10	FH893709; FH891119	**AM145618 Oryzias latipes**; Similar to vertebrate Ca++ transporting ATPase (2e-53), FM022709 cDN32 Dicentrarchus labrax cDNA (2e-40)
1092_za; 1092_ya	BAC	44-N22	FH893711; FH891121	Nasal embryonic LHRH factor (3e-06)
1093_za	BAC	37-F13	FH893708	CH211-231O6 in linkage group 21 (1e-24)
1094_za	BAC	04-F13	FH892222;	NS
1095_za; 1095_ya	BAC	05-E09	FH892431; FH889837	NS
1096_za; 1096_ya	BAC	13-L11	FH892755; FH890163	NS
1097_za; 1097_ya	BAC	33-I09	FH893056; FH890448	NS
1098_za; 1098_ya	BAC	36-H23	FH893706; FH891117	NS
1099_za; 1099_ya	BAC	45-I12	FH893712; FH891122	Tra_Liver_8_O01 similar to 60S ribosomal protein L7a (Surfeit locus protein 3)
1100_za; 1100_ya	BAC	12-E10	FH892754; FH890162	Fugu rubripes LSFR2 gene
1101_za; 1101_ya	BAC	42-N13	FH893710; FH891120	Alpha 2,6 sialyltransferase 3 [Salmo salar] (4e-17)
1102_za; 1102_ya	BAC	05-J04	FH892432; FH889838	NS

NS: No significant hits for Blast Results
Annotations in boldface are based on e-values lower than 1e-100.

Markers 0009 to 1079 represent SNP markers that were used for genotyping in multiplexed MALDI TOF assays (Sequenom).
Markers 1080 to 1102 resulted from a genomic walk using the Cumaná genomic BAC library spotted on filters.
Markers with the same number with suffixes ya or za were derived from opposite ends of the same BAC.
Markers 1103 to 1115 were generated by synteny-based candidate gene approaches.

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TABLE S1b

Primers for Sex linked markers

Marker	Origin	Forward Primer	Reverse Primer
0009	Random EST Marker	CTCATTCTGTGCTTCAACCTG	GAAATGGCCTCCAGTATCTCC
0030	Random EST Marker	TTGGTAGACCGAGACGTGAAG	TCAGCCTTGACATGAGTTACG
0032	Random EST Marker	CGATGTCAAGGCATGGTTC	TTTGCAGTGATTTCTATCATGTCC
0061	Synteny Candidate	CAGTGGTCAGCGTGAAACTC	TGGAGAAGGTACAGGTCAACG
0073	Random EST Marker	TGTAAAACGACGGCCAGTTTATCTCCACGCCTCCAATG	CAGGAAACAGCTATGACCAGTAGATGGAGGGAAGGTTGG
0090	Random EST Marker	CTTCAACCACCAAGTCAACAC	AGTGAACTCTGTCTCCATCAGC
0148	Random EST Marker	TGTAAAACGACGGCCAGTGCGCCATTTACACATACTCAC	CAGGAAACAGCTATGACCGGAGATTCAGCGGGATAAGAC
0155	Random EST Marker	GACGAGTTTCTTCCGTTTCG	CAATGTTCCTAACCAAATGTCG
0199	Random EST Marker	TCTGGGCTACTACGTCTCCAC	GACCTCACTTCTCCACGTTTC
0210	Random EST Marker	TGTAAAACGACGGCCAGTGACGAAGACAGGAAGCTGAAG	CAGGAAACAGCTATGACCAGCGAAGGACATTTCTCACAC
0228	BAC end	GTCATGTACCAAAACAGCTGCAGCCTAC	CAGCTGTCATACCACAGAGGTGTGCTC
0229	Synteny Candidate	TAAATGTCCCTGTGGTCGTGTTCGTG	TGGATGGATAATTGGTCAAAAGGGTTG
0231	BAC end	CAATCTTCTGGCTTGGACATTAAGTGAAC	GCAGAGAGAAAGAACTGGGAGGTTTCC
0244	Synteny Candidate	AGCCTGGAAGAGGATGGTCACTAAAGTC	CTTTCAGTTCTGGGTGTGTGACGTGAG
0245	Synteny Candidate	CGACAGAGGCTAAGGGAGTGTGAGG	GATAGGACAGGTTTCGAAGGATGCAGAC
0246	Synteny Candidate	CTCAGGACGCGGTCTAGAGTGGTAGG	GGAGTACTGGACCTGCTTGGATTACACC
0247	Synteny Candidate	ACGAGATGTTGTTTCGCATGGAAGAAG	CAGTAGCAAAGGTTTATTCCAGATATGGTG
0248	Synteny Candidate	GATGCCCATGTACTTGACAATCACCTC	AACTCTTCAAGGAGCCCAGCTCAAAATC
0315	BAC end	AGCTCCTTCCAATCCAAATG	AGCCGTCTCCAGTCTATTCAG
0380	BAC end	CATGATTAAGTCTATTACGCTGCAC	AGATGTGAGTGGCAACATGG
0398	BAC end	AAGCATTAGCGGTGAATCG	TTTCCTTCCCTAGATGTGTCC
0423	BAC end	TACTCCTCCGAACCCTTACG	GCTGGAAGCATATCGAACG
0429	BAC end	CGACACTAGACATAGGTAGAATGCAG	GTCCTAGAATGCGGTTAGATGTG
0442	BAC end	GTAATGAGGTTGTTGCCATGC	CAGAGGTGCTGTGTGACCTG
0490	BAC end	ACCTCACGTCTCTGGCTCTC	AGCTCTCCGCTCAGTCAATAG
0517	BAC end	GACCTGCATCCAAGGAAAG	GCCATTCATTTCTCCATGTG
0568	BAC end	CACATTTCACTGTTTATGCCAAAG	TCCCACAGGAGAGAATTACACAC

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0603	BAC end	AATGCAGGCAGATGTGGAG	TTCAGGTGGAGCCTTGTTG
0666	BAC end	GCTTGTATTCGCCGTTGTG	TTGATCTAATTTCCTCTGTGTGATG
0691	BAC end	GGACGTTCATTGCTGCTG	CATGTATCTTACTGAGCTGTACCAAC
0987	BAC end	AGCGGTCATGCACTAACAAG	CTGCCAACGAACATAAATGC
1025	BAC end	GTGGATGGGACAGAATTTATCAAC	TTCGTCTGGATGGAAGAACAG
1103	Synteny Candidate	CCTTCACTTCCTGCCATCTTC	AAACAACATCCTCACCTTCAAAC
1104	Synteny Candidate	AAAGCATCGCAACAGTGTCCCATC	CGCATGTGGATCTTGAGTTTGTCCTG
1105	Synteny Candidate	GCCTGAAGAACTACTGCGAGTGCTACG	CTCTCCTCAAAGTTCTTGCAGCCGATG
1106	Synteny Candidate	CCTCCATTTGGGTTCTTACGGATAGG	CTGGTAGGATGTATTCCATGACCCACTG
1107	Synteny Candidate	GCACTGTTTCTCCGTCGTCTTCTGAC	GGCACCTGGACAGAGTGGAGATTAGAC
1108	Synteny Candidate	TAAGGCAGGCTAGTGATGTCCCGTTTAG	GCAGCAGCAACACCATCAACTACAG
1109	Synteny Candidate	CTGTGGTGATTTCTGGACTAAGGTCTCC	GACCCCTACGAGCCAGAAGAAAGACAC
1110	Synteny Candidate	AGGTACATCTTCTCCAGGATGCCGTAAG	GCTGCGCAGAGATATAACGAGTTGTC
1111	Synteny Candidate	GCCCACATAAAGGAGCTTGAGCAGAG	CTTGAAGTCTGCCACCTCCCTCTCC
1112	Synteny Candidate	GTTCTTCTCCCCCAGCTGTCAGTCTC	CTCGATACACAGAGTCCCGAAAACGAAC
1113	Synteny Candidate	TTGTCTACGATGAGGAGTGCTTCCAG	CCTCTCGATGTGATAGGCATTGCTG
1114	Synteny Candidate	AAGAAAGCGAGCAGCGAATCCATC	GGCGCAGGAATCGACTCATACTACG
1115	Synteny Candidate	GGGAAGAAAGGAGAGAGTCACTG	CAGCAATTTCAACAGAATTACAA
1080_ya	BAC end	GAGCTCCTCTCACATCAAAATGCACAGC	GCCATACTGCTTAGGCTTTGCTTGACTG
1081_ya	BAC end	CTGCCAGTTCTTTGGTTTTTATGTGATTCC	GAAAATACAAAATCCCTCGCATCCAGTG
1082_ya	BAC end	GGCTTCCGTACCTGTGTTTCATGTTCTC	TCTTGGCAGGAATGTGCTTCTCTAGC
1082_za	BAC end	GTGGGTTCCTGTTCTGCAGCTTATCTTC	CTGTTGACATGTCTGTTGGCTGAGAGG
1083_ya	BAC end	GTCATGTACCAAAACAGCTGCAGCCTAC	CTAGACGTCACAAAAACAGGCCTTACCC
1083_za	BAC end	CAGCCCTCAGATACCTGTACATTGTTCC	GCCATACTGCTTAGGCTTTGCTTGACTG
1084_ya	BAC end	GTGGTTGGTAGATGACATCCTGTGACTG	TTAGCCTGAAACGTTACTCGGACTGG
1084_za	BAC end	CACAGAACCAATTCTGCAGTGAGC	AGGAGCAGCTGGTTCTTGATGTCTTG
1085_ya	BAC end	GGAAGAGCATCCAGACAAGACAGAAAGC	TTTCTACCTCCTGCACTTCCCTTTCCAG
1085_za	BAC end	GGTGTTTGAATAGACGGGGAAGTTGAGC	GCTAGAGTCAGCGCCACAATTCTCAC
1086_ya	BAC end	CATTACAGCAGCTGATGCGAGAAAGGTC	CCTCCACAACTGACCACGGTGTAAAAAC
1086_za	BAC end	AACGTTAGCCGAGACGCTAGACTTTGAG	GCCAGCTGACTGACAATCCAGGATAAAG
1087_ya	BAC end	CTTTTGGGATTACAGCATCCGACCAC	ACGGCCCTGAGTCAGAGAGTAATGG

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1087_za	BAC end	GTTGAGCTGCAGAGGGAATGGATAACTC	CTGTGGAAGCGGTAGGAAAGTGAATC
1088_ya	BAC end	TTTTCACGTGTGCTTAGCATTTCTCCTC	TTATATGATCGTTGCAGAGGTTCACAG
1088_za	BAC end	CTTCAAACGATTCTCCCTGACACTTCAC	CCACACTGGCAAATTAGAAACCAACATC
1089_ya	BAC end	TTCATCCTGACACAGTTATGGTTCATGG	AGAAACACACACACGCACATACACACAC
1089_za	BAC end	AGCACCATCATCTTCCATTACCTTAGCC	GCGGAGACAATAAAGTGCTCAGAGAGAG
1090_ya	BAC end	GTACATCAAGGTGTACAGACGCAGATGG	ATTGTGCGTGCACATGTCTAGTTTTACG
1090_za	BAC end	ACCGCCTGCTTATTCCAGTAATTCAGAG	ATGAATCTCTCTTCCTGGGATCTTCACC
1091_ya	BAC end	AGGAGGTCACAAAAGAAGCCAGAACAAC	CTTGATCTTCCATTGTCACACTTTCGTG
1091_za	BAC end	TCTCGTTTTGTTGTTGTGTCTCAGTGTG	GATTAGGAAGGCAGGGAGAGAAGGAAAG
1092_ya	BAC end	AACCATGCATTGACTTGCCTCATCTAAG	CTTTTGCCTTCAGATGTTTCCACAAGAG
1092_za	BAC end	GGCCGCATACCATCTAGAGTAAGCTCTG	TTTCGCTTTAATGGCCTTCAGACTTCAG
1093_za	BAC end	CTATATTTTTGTGGGTGGCAGATGATCC	CCTCTGGCCTCAAACAGAGTTTAAGTGAG
1094_za	BAC end	AGTACGTTAGCCATGAGGGTTTAGTTCG	CCTATAGAACCAGGCTCTCCACACGAG
1095_ya	BAC end	GACTGTGGCACTACGGGACTAAGGAAAG	TATACATAGCACCTCCACAGCCTCCTTC
1095_za	BAC end	GCGGAGACAATAAAGTGCTCAGAGAGAG	GCTTAGATCCAAAAGTCAGTCCCGAGAG
1096_ya	BAC end	ATACCTGACATCGCCTGTACAGATACCC	ATGCAGCGGTGCTTAGAGGAATACAGTC
1096_za	BAC end	GCACACCCCTTCTGGGGTATTTGTAAG	ACGCCTGTTCAGGAGGAGAGAGTTTTTG
1097_ya	BAC end	AGTCCGAGTGAAGGTTAGCTCTGAGGAG	TCTGCAGAGTCATACTGAAAGGGGTGAC
1097_za	BAC end	AGAAAACCTGCTAGGCCCCACTCTAATC	CCTCGTCCACTGGAAAGATGAAAGCTAC
1098_ya	BAC end	CCTGCAAGTTGTTGTTACTCAGGTCCAG	GCTCTGTAAGGTCGCACATTGTTACTGC
1098_za	BAC end	GGAGCACTGGGAAGAAAAGGATGAGTTC	CCTACCAACTATACCCATGACCCAAGAC
1099_ya	BAC end	GAGCGCTACCTTGAAGAATTCCACTCAG	TCTTAGGAGGGAAGGTATTGTCGTCCTG
1099_za	BAC end	ACTAAGCTGGAGAAGGCTAAGGCCAAG	GCCAGTCAGCCACTTACTCTCAAGTATC
1100_ya	BAC end	GTCCAGAGTTTCCGTGGTAACCAACTC	CCAACCACTGCTGATTGAGATGTCG
1100_za	BAC end	CTCGCACCAGGAAGTACTCTGATATTGG	AGTGTATTTCAGGTCTGGACCGAGTAGG
1101_ya	BAC end	TTAAAACTGGAGCGTATCTCAGCACTGG	CCCAGGAAAACATAATGTCCTGAATCAC
1101_za	BAC end	GCATGAAGAACATGTGTGGTAAATGCTTG	CTCATGCTGTAAATTGCCTCTTCCTCTG
1102_ya	BAC end	ACTTTGTGGTGGACCTTCATTTTCCTTC	ATTGAACTCCTGGAGGATCTCAGCCTTC
1102_za	BAC end	TGAGTGGGGTTGTGTATGTGTTTTTGAG	CATGGGAATTTAATCCAAGGACGAATG

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TABLE S2

Number and sex ratio of progeny in mapping crosses

	Males	Females	Sex Ratio M : F
Cross76_Cumaná_Quare			
F1	24	32	0.75
F2 from F1 Pair 1a,b	25	19	1.32
F2 from F1 Pair 4	18	15	1.20
F2 from F1 Pair 5	17	16	1.06
F2 from F1 Pair 6	11	10	1.10
F2 from F1 Pair 7	2	7	0.29
F2 from F1 Pair 8a,b	2	3	0.67
F2 from F1 Pair 9	2	2	1.00
F2 from F1 Pair 11a,b	10	12	0.83
F2 from F1 Pair 12	2	12	0.17
F2 from F1 Pair 17	15	8	1.88
Total	**128**	**136**	**0.94**
Cross99_Quare_Cumaná			
F1	6	8	0.75
F2 from F1 Pair 2	26	41	0.63
F2 from F1 Pair 3	13	26	0.50
F2 from F1 Pair 4	2	4	0.50
F2 from F1 Pair 6	42	27	1.56
F2 from F1 Pair 7	6	9	0.67
Total	**95**	**115**	**0.83**
Cross150_Quare_Cumaná			
F1	1	1	1.00
F2 from F1 Pair 1	**50**	**48**	**1.04**
Cross153_Quare_Cumaná			
F1	6	6	1.00
F2 from F1 Pair 1	39	44	0.89
F2 from F1 Pair 3	35	19	1.84
F2 from F1 Pair 4	12	18	0.67
F2 from F1 Pair 5	10	16	0.63
F2 from F1 Pair 6	44	40	1.10
Total	**146**	**143**	**1.02**

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Cross157_Quare_Cumaná

F1	24	34	0.71
F2 from F1 Pair 1	6	3	2.00
F2 from F1 Pair 2	23	27	0.85
F2 from F1 Pair 3	4	3	1.33
F2 from F1 Pair 4	32	44	0.73
F2 from F1 Pair 5	13	16	0.81
F2 from F1 Pair 6	17	19	0.89
F2 from F1 Pair 7	44	42	1.05
F2 from F1 Pair 8	21	18	1.17
F2 from F1 Pair 9	23	28	0.82
F2 from F1 Pair 10	19	35	0.54
F2 from F1 Pair 11	7	8	0.88
F2 from F1 Pair 12	12	24	0.50
F2 from F1 Pair 13	12	21	0.57
F2 from F1 Pair 14	1	5	0.20
F2 from F1 Pair 15	9	9	1.00
F2 from F1 Pair 16	3	5	0.60
F2 from F1 Pair 17	42	36	1.17
F2 from F1 Pair 18	14	10	1.40
F2 from F1 Pair 19	49	35	1.40
F2 from F1 Pair 20	26	30	0.87
F2 from F1 Pair 21	28	30	0.93
F2 from F1 Pair 22	14	23	0.61
F2 from F1 Pair 23	3	7	0.43
Total	**446**	**512**	**0.87**

Cross 158_Quare_Cumaná

Number of Individuals and sex ratios

Male phenotypic segregation

	Total	Males	Females	Sex Ratio M : F	Male phenotypes, 3 Y-linked traits present	Male phenotypes, Y-linked traits absent
F1 generation	**63**	42	21	2.00	19	22
F2 from F1 Pair 5	**94**	74	20	3.70	40	34
F2 from F1 Pair 9	**28**	24	4	6.00	13	11
F2 from F1 Pair 10	**50**	44	6	7.33	23	21
F2 from F1 Pair 11 (XX male)	**32**	16	16	1.00	0	16
F2 from F1 Pair 14 (XX male)	**13**	8	5	1.60	0	8
F2 from F1 Pair 16 (XX male)	**43**	19	24	0.79	0	19
F2 from F1 Pair 6	**15**	8	7	1.14	8	0
F2 from F1 Pair 7	**21**	11	10	1.10	11	0

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F2 from F1 Pair 15	**21**	11	10	1.10	10	1 (recombinant)
Total F2 progeny	**317**	215	102	2.11	105	109
F2 Pairs 11,14,16 (XX males)	**88**	43	45	0.96	0	43
Total	**380**	**257**	**123**	**2.03**	**124**	**131**

Note : The F1 pairs from three genotypic category of males are shaded in three colours; 87 F2 progeny were analyzed for sex chromosome recombination, see Table S4.

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TABLE S3

Polymorphic SNP markers used to distinguish between four sex chromosomes in cross 158

Distinction	Marker	♂ Parent allele	♀ Parent allele
X^CY^C / $X^{Q1}X^{Q2}$	0009	G	A
	0030	C	T
	0032	G	A
	0073	G	A
	0090	T	C
	0148	G	C
	0155	G	A
	0231	T	C
	0248	A	G
	0429	A	G
	0517	G	A
	0666	T	C
	0442	C	G
X^{Q1} / X^{Q2}	0423	C	TC
	0229	T	TC
	0315	A	AG
	0230	C	CT
	0245	C	CG
	0061	C	CT
X^C / Y^C	0210	CT	T
	0228	AG	A
	0568	AG	A
	1025	CT	C
	0490	GT	T

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TABLE S4a

Analysis of sex chromosome recombination in Pairs 11,14 &16 (with $X^{C}X^{Q2}$ F1 males) of Cross158

X or Y (gametes from F1 female or F1 male meiosis)	Number	% of total	% of rec
Total no. of gametes analyzed in F1 generation (87 x 2)	174	100	
NR (from 52 NR F2, 29 F2 with a single rec gamete (104+29)	133	76.4	
Total rec gametes (29 +12 from F2 with two rec gametes)	41	23.6	100
Rec gametes inherited from F1 female meiosis	29	16.7	70.7
Rec gametes inherited from F1 male meiosis	12	6.9	29.3

87 F2 progeny of 3 F1 pairs with female $X^{C}X^{Q1}$ and male $X^{C}X^{Q2}$ genotype were analyzed.
NR: non recombined, rec: recombined
Conclusion: Recombination between the sex chromosomes is significantly lower in XX males compared to XX females, hence dependent on phenotypic sex.

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TABLE S4b

Distribution of crossover events across the sex LG

	distal CO	non distal CO	Total
F1 male meiosis	10	2	**12**
F1 female meiosis	15	14	**29**
Total	**25**	**16**	**41**

CO: crossover. Distal refers to about one third of the LG in vicinity of the *Sex* locus, non distal is equivalent to the proximal 60% of the sex linkage group.
Conclusion: The position of the CO events along the sex chromosome is clustered towards the distal region in male meiosis. The null hypothesis that CO are equally distributed in male and female meiosis could be rejected ($p< 0.0001$).

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