

# GENETICS

## Supporting Information

<http://www.genetics.org/cgi/content/full/genetics.108.098541/DC1>

### **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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DOI: 10.1534/genetics.108.098541DOI

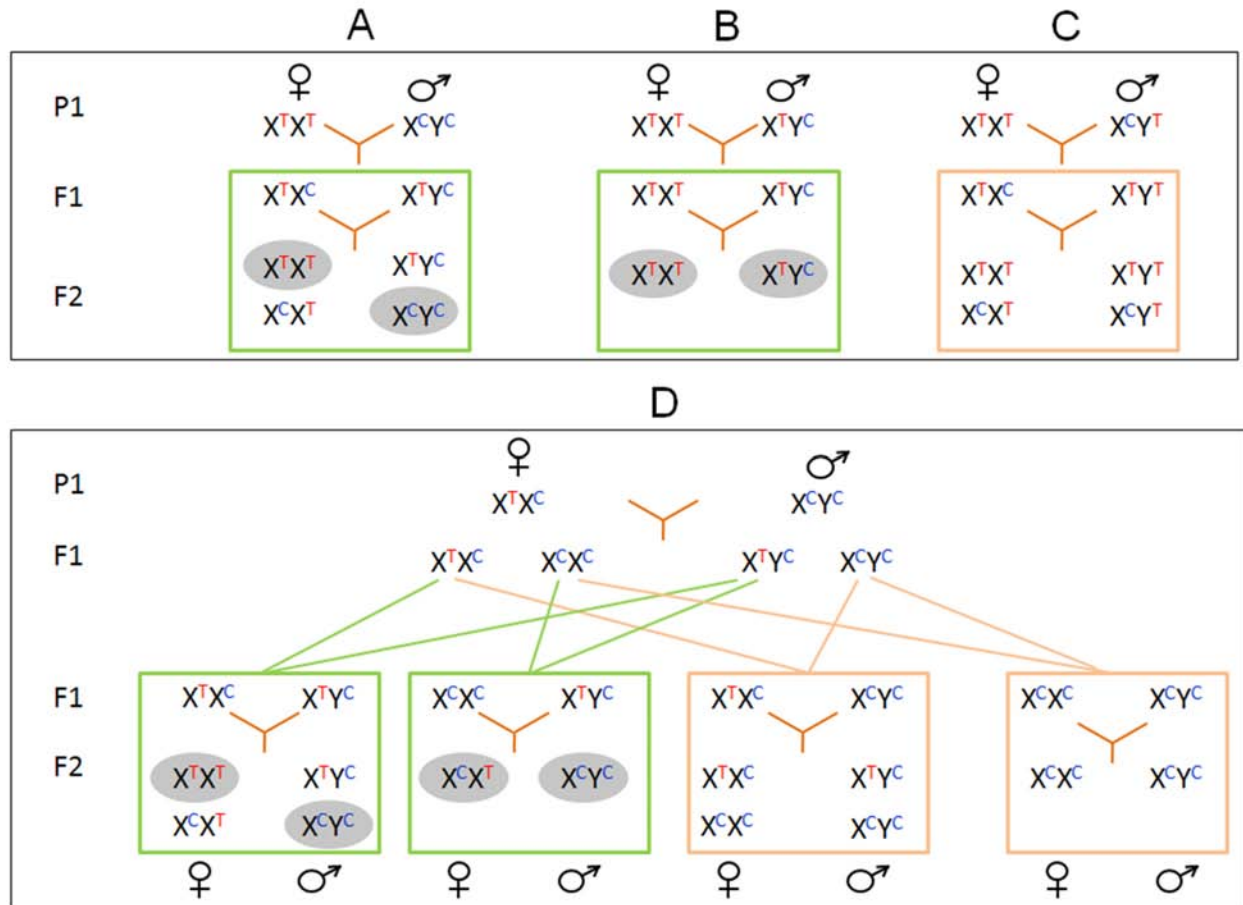


FIGURE S1.—Segregation pattern of different sex linked SNP markers in F2 population. (A) ♀ and ♂ Grandparents homozygous for opposite sex linked alleles. All ♀ and ♂ F1 offspring are heterozygous for such a marker and only 50% of the total F2 individuals (25% ♀ and 25% ♂) have distinctly sex specific 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.

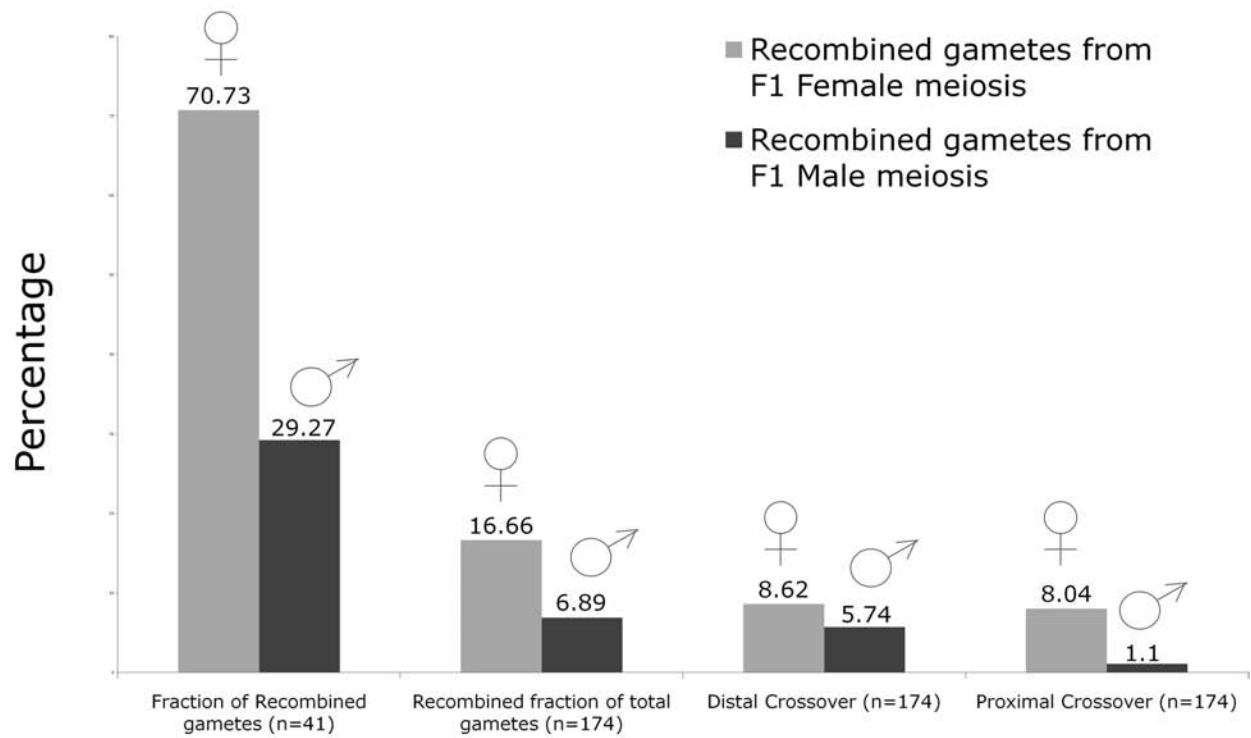


FIGURE S2.—Analysis of sex chromosome recombination frequency and distribution in F1 meiosis from cross158-Pairs11,14 and 16. In 87 F2 progeny from F1 parents with female  $X^C X^{Q1}$  and male  $X^C X^{Q2}$  genotypes 41 of 174 sex chromosomes were found to be recombined. Distal refers to 10-20% of the mapped distal end of the sex chromosome (corresponding 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)

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	<b>Fructose-1,6-bisphosphatase 1 [Danio rerio]</b>
0032	EST	Tra_Embryo_8_L19	ES386074	<b>Steroid-sensitive protein 1 [Danio rerio]</b>
0061	EST	Qua_Embryo_B2_E02	ES379217	<b>Ectodermal-neural cortex 1 ENC1</b>
0073	EST	Qua_Embryo_C1_D11	ES379375	<b>Replication factor C (activator 1) 3 [Danio rerio], CAG00421,</b> [Tetraodon nigroviridis] (Medaka Chromosome 14
0090	EST	Blu_Brain_U2-3_E08	ES372993	N-Shc (1E-50)
0148	EST	Blu_Testis_6_H06	ES380905	<b>Calmodulin regulated spectrin-associated protein 1</b>
0155	EST	Oro_Skin_3_K13	ES385036	Splicing factor, arginine/serine-rich 12 (1.2E-31)
0199	EST	Blu_Brain_L2_H11	ES372663	<b>Thyroid hormone receptor-associated protein 2</b>
0210	EST	Blu_Testis_6_K19	ES380974	<b>Heat shock 70kDa protein 5 (glucose-regulated protein 78kDa)</b>
0229	EST	Tra_Embryo_10_F17	ES374479	<b>Cyclin I</b>
0230	BAC	24-K15	FH890169	NS
0244	EST	Oro_Retina_5_C12	ES373878	<b>TGF beta-inducible nuclear protein 1 (CDK105)</b>
0245	EST	Tra_Embryo_15_F16	ES375709	<b>Catenin ARVCF [Danio rerio]; DW618591</b>
0246	EST	Tra_Embryo_2-1_F11	ES371278	<b>NADH dehydrogenase ubiquinone 1 alpha subcomplex, 8, 19kDa</b>
0247	EST	Tra_Embryo_1-4_G05	ES371208	<b>Transcription factor BTF3a</b>
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

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 <i>Oryzias latipes</i> 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	<b>aim1 slc45a2 membrane-associated transporter protein B</b>
1079	Homology cloning		FJ236234	<b>phosphodiesterase 6A</b>
1103	EST	Tra_Embryo_3-3_G01	ES371695	<b>Cyclin G2</b>
1104	Consensus	Medaka ZBTB7C <sup>s</sup>	ENSORLG00000005778	ZINC FINGER AND BTB DOMAIN CONTAINING Protein
1105	Consensus	Medaka LIN54 <sup>s</sup>	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 <i>Cyprinus carpio</i> mRNA for <b>allograft inflammatory factor-1</b>
1109	EST	Blu_Testis_8_E24	ES380544	<b>TIA1 cytotoxic granule-associated RNA binding protein</b> [Danio rerio]
1110	EST	Blu_Testis_8_P16	ES380724	<b>COP9 signalosome subunit 4</b>
1111	EST	Blu_Testis_6_H23	ES380918	<b>Restin</b> (Reed-Steinberg cell-expressed intermediate filament-associated protein)
1112	EST	Blu_Testis_4_A23	ES379967	<b>Zinc finger FYVE domain-containing endosome-associated FYVE-domain protein</b>
1113	EST	Qua_Embryo_01_C04	ES383103	<b>beta A4-crystallin</b>
1114	EST	Blu_Testis_8_G09	ES380567	<b>Putative alpha-mannosidase C1orf22</b>
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

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	<b>AM145618 <i>Oryzias latipes</i></b> ; Similar to vertebrate Ca <sup>++</sup> transporting ATPase (2e-53), FM022709 cDN32 <i>Dicentrarchus labrax</i> 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 ( <i>Surfeit locus protein 3</i> )
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 [ <i>Salmo salar</i> ] (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.

**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	Syntenly Candidate	CAGTGGTCAGCGTGAAACTC	TGGAGAAGGTACAGGTCAACG
0073	Random EST Marker	TGTA AACGACGGCCAGTTTATCTCCACGCCCTCCAATG	CAGGAAACAGCTATGACCAGTAGATGGAGGGAAGGTTGG
0090	Random EST Marker	CTTCAACCACCAAGTCAACAC	AGTGAACTCTGTCTCCATCAGC
0148	Random EST Marker	TGTA AACGACGGCCAGTGCGCCATTTACACATACTCAC	CAGGAAACAGCTATGACCCGGAGATTCAGCGGGATAAGAC
0155	Random EST Marker	GACGAGTTTCTTCCGTTTCG	CAATGTTCCCTAACCAAATGTCG
0199	Random EST Marker	TCTGGGCTACTACGTCTCCAC	GACCTCACTTCTCCACGTTTC
0210	Random EST Marker	TGTA AACGACGGCCAGTGACGAAGACAGGAAGCTGAAG	CAGGAAACAGCTATGACCAGCGAAGGACATTTCTCACAC
0228	BAC end	GTCATGTACCAAAACAGCTGCAGCCTAC	CAGCTGTACATACCACAGAGGTGTGCTC
0229	Syntenly Candidate	TAAATGTCCCTGTGGTCTGTTCGTG	TGGATGGATAATTGGTCAAAAGGGTTG
0231	BAC end	CAATCTTCTGGCTTGGACATTAAGTGAAC	GCAGAGAGAAAGAACTGGGAGGTTTCC
0244	Syntenly Candidate	AGCCTGGAAGAGGATGGTCACTAAAGTC	CTTTCAGTTCTGGGTGTGTGACGTGAG
0245	Syntenly Candidate	CGACAGAGGCTAAGGGAGTGTGAGG	GATAGGACAGGTTTCTGAAGGATGCAGAC
0246	Syntenly Candidate	CTCAGGACGCGGTCTAGAGTGGTAGG	GGAGTACTGGACCTGCTTGGATTACACC
0247	Syntenly Candidate	ACGAGATGTTGTTTCGCATGGAAGAAG	CAGTAGCAAAGGTTTATTCCAGATATGGTG
0248	Syntenly Candidate	GATGCCCATGTACTTGACAATCACCTC	AACTCTTCAAGGAGCCCAGCTCAAATC
0315	BAC end	AGCTCCTTCCAATCCAAATG	AGCCGTCTCCAGTCTATTTCAG
0380	BAC end	CATGATTAAGTCTATTACGCTGCAC	AGATGTGAGTGGCAACATGG
0398	BAC end	AAGCATTAGCGGTGAATCG	TTTCCTTCCCTAGATGTGTCC
0423	BAC end	TACTCCTCCGAACCCCTACG	GCTGGAAGCATATCGAACG
0429	BAC end	CGACACTAGACATAGGTAGAATGCAG	GTCCTAGAATGCGGTTAGATGTG
0442	BAC end	GTAATGAGGTTGTTGCCATGC	CAGAGGTGCTGTGTGACCTG
0490	BAC end	ACCTCACGTCTCTGGCTCTC	AGCTCTCCGCTCAGTCAATAG
0517	BAC end	GACCTGCATCCAAGGAAAG	GCCATTCAATTTCTCCATGTG
0568	BAC end	CACATTTCACTGTTTATGCCAAAG	TCCCACAGGAGAGAATTACACAC

0603	BAC end	AATGCAGGCAGATGTGGAG	TTCAGGTGGAGCCTTGTG
0666	BAC end	GCTTGTATTTCGCCGTTGTG	TTGATCTAATTTCCCTCTGTGTGATG
0691	BAC end	GGACGTTTCATTGCTGCTG	CATGTATCTTACTGAGCTGTACCAAC
0987	BAC end	AGCGGTCATGCACTAACAAG	CTGCCAACGAACATAAATGC
1025	BAC end	GTGGATGGGACAGAATTTATCAAC	TTCGTCTGGATGGAAGAACAG
1103	Syntenly Candidate	CCTTCACTTCCTGCCATCTTC	AAACAACATCCTCACCTTCAAAC
1104	Syntenly Candidate	AAAGCATCGCAACAGTGTCCCATC	CGCATGTGGATCTTGAGTTTGTCCCTG
1105	Syntenly Candidate	GCCTGAAGAACTACTGCGAGTGCTACG	CTCTCCTCAAAGTTCTTGCAGCCGATG
1106	Syntenly Candidate	CCTCCATTTGGGTTCTTACGGATAGG	CTGGTAGGATGTATTCCATGACCCACTG
1107	Syntenly Candidate	GCACTGTTTCTCCGTCGTCTTCTGAC	GGCACCTGGACAGAGTGGAGATTAGAC
1108	Syntenly Candidate	TAAGGCAGGCTAGTGATGTCCCGTTTAC	GCAGCAGCAACACCATCAACTACAG
1109	Syntenly Candidate	CTGTGGTGATTTCTGGACTAAGGTCTCC	GACCCCTACGAGCCAGAAGAAAGACAC
1110	Syntenly Candidate	AGGTACATCTTCTCCAGGATGCCGTAAG	GCTGCGCAGAGATATAACGAGTTGTC
1111	Syntenly Candidate	GCCCACATAAAGGAGCTTGAGCAGAG	CTTGAAGTCTGCCACCTCCCTCTCC
1112	Syntenly Candidate	GTTCTTCTCCCCAGCTGTGAGTCTC	CTCGATACACAGAGTCCCGAAAACGAAC
1113	Syntenly Candidate	TTGTCTACGATGAGGAGTGCTTCCAG	CCTCTCGATGTGATAGGCATTGCTG
1114	Syntenly Candidate	AAGAAAGCGAGCAGCGAATCCATC	GGCGCAGGAATCGACTCATACTACG
1115	Syntenly Candidate	GGGAAGAAAGGAGAGAGTCACTG	CAGCAATTTCAACAGAATTACAA
1080_ya	BAC end	GAGCTCCTCTCACATCAAAATGCACAGC	GCCATACTGCTTAGGCTTTGCTTGACTG
1081_ya	BAC end	CTGCCAGTTCTTTGGTTTTTATGTGATTCC	GAAAATACAAAATCCCTCGCATCCAGTG
1082_ya	BAC end	GGCTTCCGTACCTGTGTTTTTCATGTTCTC	TCTTGGCAGGAATGTGCTTCTCTAGC
1082_za	BAC end	GTGGGTTCTGTCTGTCAGCTTATCTTC	CTGTTGACATGTCTGTTGGCTGAGAGG
1083_ya	BAC end	GTCATGTACCAAAAACAGCTGCAGCCTAC	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	GGTGTGTTGAATAGACGGGGAAGTTGAGC	GCTAGAGTCAGCGCCACAATTCTCAC
1086_ya	BAC end	CATTACAGCAGCTGATGCGAGAAAGGTC	CCTCCACAACCTGACCACGGTGTAAAAAC
1086_za	BAC end	AACGTTAGCCGAGACGCTAGACTTTGAG	GCCAGCTGACTGACAATCCAGGATAAAG
1087_ya	BAC end	CTTTTGGGATTACAGCATCCGACCAC	ACGGCCCTGAGTCAGAGAGTAATGG



1087_za	BAC end	GTTGAGCTGCAGAGGGAATGGATAACTC	CTGTGGAAGCGGTAGGAAAGTGAATC
1088_ya	BAC end	TTTTACGTGTGCTTAGCATTCTCCTC	TTATATGATCGTTGCAGAGGTTACACAG
1088_za	BAC end	CTTCAAACGATTCTCCCTGACACTTCAC	CCACACTGGCAAATFAGAAACCAACATC
1089_ya	BAC end	TTCATCCTGACACAGTTATGGTTCATGG	AGAAACACACACACGCACATACACACAC
1089_za	BAC end	AGCACCATCATCTTCCATTACCTTAGCC	GCGGAGACAATAAAGTGCTCAGAGAGAG
1090_ya	BAC end	GTACATCAAGGTGTACAGACGCAGATGG	ATTGTGCGTGCACATGTCTAGTTTTACG
1090_za	BAC end	ACCGCCTGCTTATTCCAGTAATTCAGAG	ATGAATCTCTCTTCCCTGGGATCTTCACC
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	CCTCTGGCCTCAAACAGAGTTAAGTGAG
1094_za	BAC end	AGTACGTTAGCCATGAGGGTTTAGTTCG	CCTATAGAACCAGGCTCTCCACACGAG
1095_ya	BAC end	GACTGTGGCACTACGGGACTAAGGAAAG	TATACATAGCACCTCCACAGCCTCCTTC
1095_za	BAC end	GCGGAGACAATAAAGTGCTCAGAGAGAG	GCTTAGATCCAAAAGTCAGTCCCAGAGAG
1096_ya	BAC end	ATACCTGACATCGCCTGTACAGATACCC	ATGCAGCGGTGCTTAGAGGAATACAGTC
1096_za	BAC end	GCACACCCCTTCTGGGGTATTTGTAAG	ACGCCTGTTTCAGGAGGAGAGAGTTTTTG
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	GAGCGCTACCTTGAAGAATTCCTACTCAG	TCTTAGGAGGGAAGGTATTGTGCTCCTG
1099_za	BAC end	ACTAAGCTGGAGAAGGCTAAGGCCAAG	GCCAGTCAGCCACTTACTCTCAAGTATC
1100_ya	BAC end	GTCCAGAGTTTCCGTGGTAACCAACTC	CCAACCACTGCTGATFAGATGTCTG
1100_za	BAC end	CTCGCACCAGGAAGTACTCTGATATTGG	AGTGTATTTTCAGGTCTGGACCGAGTAGG
1101_ya	BAC end	TTAAAACCTGGAGCGTATCTCAGCACTGG	CCCAGGAAAACATAATGTCTGAATCAC
1101_za	BAC end	GCATGAAGAACATGTGTGGTAAATGCTTG	CTCATGCTGTAAATTGCCTCTTCCCTCTG
1102_ya	BAC end	ACTTTGTGGTGGACCTTCATTTTCCTTC	ATTGAACCTCCTGGAGGATCTCAGCCTTC
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
<b>Cross76_Cumaná_Quare</b>			
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	<b>128</b>	<b>136</b>	<b>0.94</b>
<b>Cross99_Quare_Cumaná</b>			
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	<b>95</b>	<b>115</b>	<b>0.83</b>
<b>Cross150_Quare_Cumaná</b>			
F1	1	1	1.00
F2 from F1 Pair 1	<b>50</b>	<b>48</b>	<b>1.04</b>
<b>Cross153_Quare_Cumaná</b>			
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	<b>146</b>	<b>143</b>	<b>1.02</b>

**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	<b>446</b>	<b>512</b>	<b>0.87</b>

**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	<b>63</b>	42	21	2.00	19	22
F2 from F1 Pair 5	<b>94</b>	74	20	3.70	40	34
F2 from F1 Pair 9	<b>28</b>	24	4	6.00	13	11
F2 from F1 Pair 10	<b>50</b>	44	6	7.33	23	21
F2 from F1 Pair 11 (XX male)	<b>32</b>	16	16	1.00	0	16
F2 from F1 Pair 14 (XX male)	<b>13</b>	8	5	1.60	0	8
F2 from F1 Pair 16 (XX male)	<b>43</b>	19	24	0.79	0	19
F2 from F1 Pair 6	<b>15</b>	8	7	1.14	8	0
F2 from F1 Pair 7	<b>21</b>	11	10	1.10	11	0

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

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.

**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
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

**TABLE S4a****Analysis of sex chromosome recombination in Pairs 11,14 & 16 (with  $X^{CXQ2}$  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  $X^{CXQ1}$  and male  $X^{CXQ2}$  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.

**TABLE S4b****Distribution of crossover events across the sex LG**

	distal CO	non distal CO	Total
F1 male meiosis	10	2	<b>12</b>
F1 female meiosis	15	14	<b>29</b>
Total	<b>25</b>	<b>16</b>	<b>41</b>

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 ( $p < 0.0001$ ).