S1 Text

Supplemental Methods

Identifying Putative Genotype Errors in Prior to Sex-Linkage Analysis

Genotyping errors inflate linkage map lengths, and reduce power to infer genotype–phenotype associations. These errors can be caused by incomplete digestion of DNA during RRGS library preparation, polymorphism at restriction enzyme cut sites, and low or allelic bias in sequencing coverage [1–3]. We attempted to reduce the effects of genotyping errors by inspecting three consecutive offspring genotypes in up to a 5 Mb window, resolving the sex-specific parental haplotypes in each window, and setting offspring genotypes to missing data that could only be explained by a double recombination event, under the assumption that double recombination events are unlikely within 5 Mb windows.

To accomplish this, we first assigned variants in offspring genotypes to each parent. For example, if the genotype of an offspring was T/A, the mother was A/T, and the father was A/A, we can infer that the T was maternally inherited and that the A was paternally inherited. Then, in groups of three markers spanning no more than 5 Mb (and spaced at least 5 Kb apart from each other, randomly discarding extra SNPs within 5 Kb windows), we scored the frequencies of multilocus haplotypes that were inherited from each parent. We assumed that the non-recombined haplotypes would be more frequent in the offspring than recombined haplotypes, and thus reflect the parental gametic phases. For example, the phases of a set of three markers might be inferred to be ACT/TGC in the mother, and TGC/TGC in the father. In the offspring, we then identified non-recombined haplotypes (ACT, TGC), single recombination haplotypes (AGC, TCT, ACC, TGT), and double recombination haplotypes (AGT, TCC).

It is possible that setting suspected genotype errors to missing data could lead to underestimated map lengths by removing any real double recombination events. However, these events are probably infrequent and the effect therefore is expected to be modest. As well, our interest is primarily in comparing map lengths between the sexes, and we treated both maternal and paternal sets of markers identically, so any underestimation of recombination rates is likely to similarly affect both.

Supplemental Results

In the Ghana west family Family 1 after FDR correction, five paternal SNPs had a significant association with offspring sex; all of these were located on one end of chromosome 7. Genome-wide heterozygosity was similar in the parents of Family 1, which suggests that the power to detect female-linked and male-linked variants is similar. 2,857 sites were heterozygous in the father, 2,834 were heterozygous in the mother, and 1,660 were heterozygous in both parents. In Ghana east Family 2, three paternal SNPs were identified that were significantly associated with offspring sex, but none of these were significant after FDR correction (this cross included far fewer offspring; Fig. 4). These three SNPs were in a similar locations (within 1.6 Mb) to the sex-linked sites in Family 1 (Fig. 4). Other chromosomes (Fig. S3) did not have any variants with a significant association with sex for Family 1, but in Family 2 there were putative false positives (prior to FDR correction) on chromosomes 3 and 5 (Fig. S3). Genome-wide, there were 2447 paternal heterozygous SNPs, 2700 maternal heterozygous SNPs, and 2197 sites that were heterozygous in both parents of Family 2.

In the Ghana west Family 1, one of the five significantly sex-linked SNPs (at position 9,149,465 in *X. tropicalis* genome assembly version 10 (v10) was also 100% sex-linked (heterozygous in all sons, homozygous in all daughters), and the variants near this site (positions 8,104,283 – 13,579,674) were very strongly, but not completely sex linked. In Ghana east Family 2, all three of the significantly sex-linked SNPs were 100% linked with sex (positions 2,679,758, 5,227,940, 6,542,981). Differences in p-values between these three SNPs in Fig. 4 are a consequence of differing numbers of missing offspring genotypes. The distinctive statistical signatures of sex-linkage between the Ghana west Family 1 and Ghana east Family 2 (significant after FDR correction for the former, but not for the latter) is a consequence of the different number of offspring analyzed (Fig. 2).

Sex-linked regions in *Xenopus mellotropicalis*

A closely related allotetraploid species, *X. mellotropicalis*, has almost completely female-linked variation in the *or8h1* gene [4]. This locus maps to position 11,962,496–11,963,464 on chromosome 7 in v10 (3,679,275 – 3,680,243 in v9), which is very close to the third most strongly female-linked genomic region in *X. tropicalis* (linkage group super_352:0; positions 3,272,993 – 3,436,880 in v9; LOD score: 12.60676032) [5]. Similarly, a scaffold from the genomee of *X. mellotropicalis* with a completely sex-linked SNP corresponds to positions 250,772 —251,327 on chromosome 7 in v10 (scaffold_622, positions 39,157 – 38,626 in v9). Both of these female-linked variants in *X. mellotropicalis* are homologous to portions of the the 95% Bayes credible interval (positions 0 – 3,906,563 in v9) for the W-linked female determining marker in *X. tropicalis* [5]. As discussed in the Introduction, this supports the proposal that female heterogamy is a shared ancestral condition in *X. tropicalis* and *X. mellotropicalis*, and that the Y chromosome of *X. tropicalis* is younger than and derived from the W or Z chromosomes in this species.

Pairwise nucleotide polymorphism in expressed transcripts

We initially expected WW offspring to have no polymorphism in sex-linked transcripts because both of these W chromosomes descend from specimen BJE4362, who was the father and the grandfather of offspring of Family 3 (Fig. 2), but this expectation was not borne out (Fig. 5). We suspect that this is a consequence of mapping error, for example due to repetitive regions in untranslated regions of these transcripts. Another non-exclusive possibility is that recombination occurred in the distal portion of the sex-linked region of the W and Z chromosomes during oogenesis in the mother or in distal portion of the sex-linked region of the W and Y chromosomes during spermatogenesis in the father/grandfather. To test these possibilities, we separately quantified polymorphism in transcripts encoded by genes <6 Mb on chromosome 7 in v10 and between 6 Mb and 11 Mb of the sex-linked region (Fig. S4). If recombination occurs between the W and Y sex chromosomes (in the father of Family 3) or between the W and Z chromosomes (in the mother of Family 3), we expected diversity of the first half would be similar to levels seen in other recombining regions of the genome (i.e. the pseudoautosomal and autosomal regions). However, within three of the four sex chromosome genotypes, the first portion and second portions of the sex-linked region had similar diversities and these diversity levels were generally not similar to the pseudoautosomal and autosomal regions (see below). In the putative ZY individuals, the diversity in the 5' portion of the sex-linked region was similar to that in the autosomal region. We suspect that recombination between the Z and Y chromosomes causes polymorphism to be similar in the first half of the sex-linked region. We therefore favor a technical explanation for the inference of polymorphism in these

putative WW individuals over the biological explanation associated with recombination between W chromosome and the Y or Z chromosomes (Fig. 5).

We had no expectation about the relative level of pairwise nucleotide polymorphism in expressed transcripts in recombining compared to non-recombining regions because we do not know the proportion of sex-linked transcripts that are only expressed on one sex chromosome (and thus completely homozygous, which decreases polymorphism compared to expressed non-sex linked transcripts) or the level of divergence between expressed transcripts on different sex chromosomes (which generates heterozygous genotypes and increases polymorphism compared to expressed non-sex linked transcripts). Moreover, the level of divergence between non-recombining portions of sex chromosomes need not be constant throughout the non-recombining region [6]. The average polymorphism of expressed non-sex-linked transcripts was 0.013 (range: 0.012-0.015). That this level of polymorphism is lower than that of expressed sex-linked transcripts in putative WZ females and WY males argues for the presence of diverged sites between the sex-linked regions of the W and Z chromosomes and the W and Y chromosomes.

Reference Bias

One possible concern for these analyses relates to the possibility that reference bias – when the reference sequence influences genotypes or mapping of transcripts – could affect our results. The effect of reference bias is typically evidenced by poor mapping or inferences of lower expression in the heterogametic sex when data are mapped to a reference genome from the homogametic sex (e.g., [7]). In the current study, however, expression levels were assessed using a transcriptome assembly we generated *de novo*, and the genomic locations of assembled transcripts was assessed by mapping these transcripts to the genome assembly from a female individual that may have been heterogametic (WZ) or homogametic (WW). In several analyses using the entire data sets or subsets with specific sex chromosome genotypes, we consistently observed an excess of male-biased expression of sex-linked transcripts in heterogametic males compared to females (Figs. S6, S7, S8). For these reasons we believe our inference of extensive male-biased expression of transcripts in the sex-linked region has a biological rather than a technical basis.

Because the *X. tropicalis* individual that was used for genome sequencing was female, Y chromosome specific sequences or genes, might not be present in this genome sequence. We therefore searched for transcripts with male-specific expression in the tadpole gonad/mesonephros transcriptome that did not have a strong mapping to v10. Only one unmapped transcript had substantial male-specific expression (TRINITY_DN3464_c0_g2_i2). However, we were able to amplify and sequence a portion of this transcript in both sexes, which indicates that the gene encoding this transcript is not specific to the Y chromosome, although we did identify a diverged position between one male and one female sequence (several other individuals failed to amplify or sequence). As discussed above, other factors such as GC content and repetitive sequences also may have contributed to mapping error of transcripts to the genome assembly or of RNAseq reads to the transcriptome assembly. However, we have no reason to suspect GC content would disproportionately affect mapping in certain genomic regions (e.g., sex-linked and non-sex-linked) or in a specific sex.

Expression Bias in Offspring subsets from Family 3

If the inferred sex chromosome genotypes of the parents of Family 3 discussed in the main text are correct, we predicted that patterns of male-biased expression might be distinctive if we reanalyzed the data using a subset of individuals (subset 1) that

included only putative WW females (XT3, XT9, XT11, XT20) and only putative WY males (XT7, XT8) as compared to a subset (subset 2) that included only putative WZ females (XT2, XT6, XT10, XT16, XT17) and only putative ZY males (XT1, XT13, XT19) because females do not have a Z chromosome in the first subset but they do in the second subset. As expected, more significantly male-biased transcripts were identified in the analysis of subset 1 (Fig. S6) than subset 2 (Fig. S7), even though subset 1 included fewer individuals of both sexes. Another interesting finding that emerges from these analyses is that the portion of chromosome 7 with the high density of genes with sex-biased expression extends slightly beyond the sex-linked region that we identified when all individuals were included for subset 1, but not for subset 2. This suggests that the degeneration of the W chromosome may extend beyond 11Mb, but the male-biased expression associated with this degeneracy on the W chromosome in this region is not as apparent in WZ females due to the presence of the Z chromosome.

When we performed an analysis of differential expression between putative WW females and putative ZY males (subset 3), the results were similar to the subset 1 analysis in terms of number and identities of differentially expressed transcripts in and near the sex-linked region (Fig. S8). We also performed an analysis of only putative WZ females and putative WY males (subset 4). If male-biased expression were solely due to degeneration on the W chromosome, we expected this comparison to have even more modest sex-differences than the other subsets. However, the pattern of male biased expression was relatively similar to the analysis of subset 2 (which is putatively WZ females versus ZY males; Fig. S9). This suggests that, in addition to degeneration of the W chromosome, there may be regulatory differences of alleles on the sex-linked portions of the Z and Y chromosomes that contribute to male-biased expression in the main analysis (Fig. 6). Alternatively or in addition, regulation of several genes in the sex-linked portion of the Z chromosome may vary depending on whether it this chromosome is in a female or male individual.

Table A. Genes on chromosome 7 between 0–11 Mb in v10 genome assembly with significantly male or female biased expression in gonad/mesenephros tissue of stage 50 tadpoles. Information includes transcript ID (TransID), the Start and End coordinates in v10, annotation, log male/female fold change (logFC), the p-value after false detection rate correction (FDR), and the nature of the biased expression (Bias), whether male or female, and whether or not the transcript was sex-specific (Sex-specific).

TransID	Start	End	Annotation	logFC	FDR	Bias	Sex-specific
TRINITY_DN4289_c0_g1_i1	1075164	1075454	add3 (adducin 3) - intronic	5.388	0.012	male	yes
TRINITY_DN3468_c0_g1_i5	3172165	3172943	tcf7l2 (transcription factor 7 like 2)	4.993	0.002	male	yes
TRINITY_DN3640_c1_g1_i1	3721434	3721778	no annotation	4.245	0.007	male	no
TRINITY_DN80732_c0_g1_i1	3723091	3723431	no annotation	4.045	0.024	male	no
TRINITY_DN43271_c0_g2_i1	3724073	3725113	LOC101733565 (pleckstrin homology domain-containing family S member 1-like)	5.64	0	male	yes
TRINITY_DN43271_c0_g1_i3	3725107	3725831	LOC101733565 (pleckstrin homology domain-containing family S member 1-like)	4.126	0.027	male	no
TRINITY_DN43271_c0_g1_i1	3725814	3727694	LOC101733565 (pleckstrin homology domain-containing family S member 1-like)	6.344	0	male	no
TRINITY_DN151694_c0_g1_i1	3742509	3742866	no annotation	5.06	0.001	male	yes
TRINITY_DN56662_c0_g1_i1	3806825	3807132	no annotation	4.283	0.039	male	yes
TRINITY_DN5848_c0_g1_i2	3970144	3989868	vwa2 (von Willebrand factor A domain containing 2)	9.329	0	male	yes
TRINITY_DN452_c2_g1_i2	5292703	5293449	shtn1 (shootin 1)	5.254	0.012	male	yes
TRINITY_DN3679_c0_g1_i3	5406344	5407384	no annotation	5.625	0	male	yes
TRINITY_DN1115_c0_g1_i18	7020737	7051446	pzp (pregnancy-zone protein)	-8.446	0.032	female	yes
TRINITY_DN16274_c0_g1_i4	7551805	7776967	multiple	5.308	0.01	male	yes
TRINITY_DN19099_c0_g1_i1	7631286	7631552	LOC100487578 (alpha-2-macroglobulin-like protein 1) - intronic	4.984	0.011	male	yes
TRINITY_DN42788_c0_g1_i1	7633454	7633953	multiple	5.388	0.001	male	yes
TRINITY_DN2504_c0_g2_i3	7635900	7885430	multiple	9.723	0	male	yes
TRINITY_DN102758_c0_g1_i1	7651629	7652058	LOC100487578 (alpha-2-macroglobulin-like protein 1) - intronic	4.247	0.001	male	no
TRINITY_DN101318_c0_g2_i2	7759551	7764141	LOC100491357 (ovostatin-like)	3.955	0.031	male	no
TRINITY_DN1080_c0_g1_i7	7775636	7776317	LOC108644460 (murinoglobulin-2-like)	6.643	0	male	yes
TRINITY_DN1080_c0_g1_i4	7976928	7994749	phc1 (polyhomeotic homolog 1)	10.269	0	male	yes
TRINITY_DN1080_c0_g1_i3	7982811	8134828	multiple	8.553	0	male	yes
TRINITY_DN2746_c0_g1_i10	7998146	8009403	m6pr (mannose-6-phosphate receptor (cation dependent))	4.75	0.003	male	no
TRINITY_DN20224_c0_g1_i1	7998819	7999821	m6pr (mannose-6-phosphate receptor (cation dependent)) - intronic	5.269	0.001	male	yes
TRINITY_DN2746_c0_g1_i4	8006123	8009403	m6pr (mannose-6-phosphate receptor (cation dependent))	6.223	0.001	male	no
TRINITY_DN4521_c7_g1_i1	8133674	8134175	LOC100488897 (ovostatin)	4.958	0	male	no
TRINITY_DN32117_c0_g1_i1	8524642	8525266	LOC116412135 (ovostatin-like) - intronic	4.033	0.02	male	no
TRINITY_DN14386_c0_g4_i2	8664301	8665021	no annotation	3.596	0.015	male	no
TRINITY_DN18403_c0_g1_i9	8686743	8721352	ovos2 (alpha-2-macroglobulin like 1 pseudogene)	4.391	0.001	male	no
TRINITY_DN1430_c1_g2_i3	9452410	9453238	atn1 (atrophin 1)	9.212	0	male	yes
TRINITY_DN26991_c0_g2_i1	9584961	9587403	LOC116412097 (phosphatidylglycerol–prolipoprotein diacylglyceryl transferase-like)	3.442	0.017	male	no
TRINITY_DN20482_c0_g1_i4	9603480	9604077	no annotation	2.834	0.014	male	no
TRINITY_DN5596_c0_g1_i3	9675146	9681174	LOC100127624 (provisional ortholog of tumor protein p63 regulated 1 like)	9.81	0	male	yes
TRINITY_DN3866_c0_g1_i11	10006830	10008040	gapdh (glyceraldehyde-3-phosphate dehydrogenase)	8.856	0	male	yes
TRINITY_DN3866_c0_g1_i10	10006830	10008040	gapdh (glyceraldehyde-3-phosphate dehydrogenase)	7.807	0.001	male	yes
TRINITY_DN3866_c0_g1_i7	10006841	10008366	gapdh (glyceraldehyde-3-phosphate dehydrogenase)	7.166	0.006	male	no
TRINITY_DN3866_c0_g1_i5	10008064	10008773	gapdh (glyceraldehyde-3-phosphate dehydrogenase)	8.072	0	male	yes
TRINITY_DN29119_c0_g1_i1	10008933	10009139	gapdh (glyceraldehyde-3-phosphate dehydrogenase)	5.387	0	male	no
TRINITY_DN328_c1_g1_i7	10009087	10011410	gapdh (glyceraldehyde-3-phosphate dehydrogenase)	9.381	0	male	yes
TRINITY_DN328_c1_g1_i6	10010171	10010432	gapdh (glyceraldehyde-3-phosphate dehydrogenase) - intronic	6.254	0	male	no
TRINITY_DN7512_c0_g1_i1	10011106	10011339	gapdh (glyceraldehyde-3-phosphate dehydrogenase) - intronic	4.052	0.012	male	no
TRINITY_DN21880_c0_g1_i1	10137392	10137732	ltbr (lymphotoxin beta receptor)	6.559	0	male	yes
TRINITY_DN12616_c0_g1_i1	10137920	10138154	ltbr (lymphotoxin beta receptor)	5.114	0.001	male	yes
TRINITY_DN23111_c0_g1_i1	10138245	10138551	ltbr (lymphotoxin beta receptor)	6.084	0	male	yes
TRINITY_DN2118_c0_g1_i8	10138785	10146504	ltbr (lymphotoxin beta receptor)	8.535	0	male	yes
TRINITY_DN21513_c0_g1_i6	10147293	10148127	ltbr (lymphotoxin beta receptor)	5.152	0.014	male	yes

Table B. Number of transcripts that had significantly male or female biased expression in gonad/mesenephros tissue of stage 50 *X. tropicalis* tadpoles. The numbers in parenthesis correspond to the number of sex-specific transcripts – where expression was detected exclusively in male or female offspring.

chromosome	male	female
Chr1	9 (3)	2 (1)
Chr2	4 (2)	1 (0)
Chr3	9 (2)	27 (18)
Chr4	7 (2)	4 (2)
Chr5	10 (3)	1 (0)
Chr6	2 (2)	2 (0)
Chr7	50 (29)	4 (3)
Chr8	11 (1)	3 (2)
Chr9	2 (1)	0 (0)
Chr10	3 (1)	0 (0)
scaffolds	1 (1)	0 (0)
unmapped	3 (2)	2 (0)

Table C. Genes on chromosome 3 between 114–128 Mb in v10 genome assembly that had significantly male or female biased expression in gonad/mesenephros tissue of stage 50 tadpoles. Column names follow Table A

TransID	Start	End	Annotation	logFC	FDR	Bias	Sex-specific
TRINITY_DN11483_c0_g1_i2	114682108	114700112	arrdc4 (arrestin domain containing 4)	-9.265	0.003	female	yes
TRINITY_DN4919_c0_g1_i4	115896740	115905228	selenos (selenoprotein S)	-10.852	0	female	no
TRINITY_DN4919_c0_g1_i2	115896740	115905228	selenos (selenoprotein S)	-6.521	0.016	female	no
TRINITY_DN20315_c2_g1_i2	116754955	116756417	Xetrov90009290m.g	-8.776	0.004	female	yes
TRINITY_DN1544_c0_g1_i4	117686570	117687358	calml4 (calmodulin like 4)	-8.675	0.002	female	yes
TRINITY_DN1544_c0_g1_i8	117686570	117695154	calml4 (calmodulin like 4)	-9.076	0.006	female	yes
TRINITY_DN1544_c0_g1_i2	117686570	117695154	calml4 (calmodulin like 4)	-10.894	0.001	female	yes
TRINITY_DN1544_c0_g1_i3	117686570	117701646	overlap calml4 and cln6	-9.332	0.003	female	yes
TRINITY_DN4169_c0_g1_i3	117960573	117963031	no annotation	-8.671	0.004	female	yes
TRINITY_DN238_c0_g2_i2	117998782	117999627	LOC108646189 (uncharacterized) - intronic	-9.242	0.003	female	yes
TRINITY_DN2503_c0_g1_i9	122034576	122049371	c2orf42 (chromosome 2 open reading frame 42)	5.01	0.014	male	no
TRINITY_DN2503_c0_g1_i12	122034576	122049371	c2orf42 (chromosome 2 open reading frame 42)	-7.948	0.009	female	no
TRINITY_DN826_c0_g2_i2	122371153	122374651	gmcl1 (germ cell-less - spermatogenesis associated 1)	-9.701	0.005	female	yes
TRINITY_DN7881_c0_g1_i2	123083266	123116702	slc23a2 (solute carrier family 23 (ascorbic acid transporter) member 2)	-9.422	0.028	female	yes
TRINITY_DN7881_c0_g1_i11	123108848	123116702	slc23a2 (solute carrier family 23 (ascorbic acid transporter) member 2)	-9.75	0.025	female	yes
TRINITY_DN2665_c0_g1_i1	125499506	125505708	purb (urine-rich element binding protein B)	-12.417	0.008	female	yes
TRINITY_DN2665_c0_g1_i2	125501145	125505708	purb (urine-rich element binding protein B)	-6.722	0.005	female	no
TRINITY_DN18664_c0_g1_i3	125663361	125664448	vamp8 (vesicle associated membrane protein 8)	-8.133	0.022	female	yes
TRINITY_DN1473_c0_g1_i4	126062381	126065503	slc20a1 (solute carrier family 20 member 1)	-12.632	0.002	female	yes
TRINITY_DN3202_c0_g1_i1	126356125	126368512	ogdh (oxoglutarate dehydrogenase)	-10.513	0.017	female	yes
TRINITY_DN3202_c0_g1_i14	126356125	126372002	ogdh (oxoglutarate dehydrogenase)	-10.681	0.001	female	no
TRINITY_DN4235_c0_g1_i7	127122736	127146974	entpd4 (ectonucleoside triphosphate diphosphohydrolase 4)	-9.556	0.028	female	no

Table D. Primer pairs (in rows) used in this study. Forward and reverse sequences are given in 5' to 3' orientation.

Locus	Forward name	Forward sequence	Reverse name	Reverse sequence
-	scaf835_16643_f	TATTTATTAACGCCATCATTAAAGG	scaf835_17422_r	CTTGTGGCGCAGCAGAAA
-	scaf7931_1217_f	GATTAAAGGCGTGGTCTGGA	scaf7931_2097_r	AACGAACGACAAAGGGAAAA
vwa2	Xt_vwa2_UTR_F1	GGAAACGGAGGCTGAAAACC	Xt_vwa2_UTR_R2	TGTGCTGATTTTCCTGGGGC
bag3	scaf8518_802_f	CCAGCAATGATATAGGGAGTCAG	scaf8518_1565_r	GGAAGGGGCTTTTGACTTTC
LOC108644867	LOC108644867_f1	GGTCCCAAGCATTCTGGATA	LOC108644867_r1	AAGAGAGAGTGCCGCAGAAG
phc1	scaf2_150071042_f	GATTGGTCCCCTTCCGTAAT	scaf2_150071793_r	GGATTTCAGGCCAGCAGTTA
LOC100488897	Scaf2_f1	TGGGCATTTGGAGGGGMATC	Scaf2_r2	CCTGCAGCTCGACTTCTTCA
aicda	aicda_f1	ATTGAATTGCTGAGCCGGTA	aicda_r1	TTTTCTGATCGCCATTAGGG
LOC116406517	trop_east_SNP1_F1	ACAAAGCCGTGGGAGCAGTT	trop_east_SNP1_R2	CCGGGGCTTGAGTTCCTATA
	trop_east_SNP1_F2	GACGTGATGGTTCCAGATAG	trop_east_SNP1_r1	TCCCCATTTCCTGCTTGTGG
LOC100127624	trpg1l-like_f1	GTTCTGCCTGGTTTGTGGAT	trpg1l-like_r1	TCCCCCGGCTTAAATATACC
grp162	grp162_f1	CCCAAGGTAAACACCCCTTT	grp162_r1	TATGGCAACCCTTTGTAGGC
LOC116412229*	Xetrov90017422_f1	CCCAGCAGCTCAGCTACCTA	Xetrov90017422_r1	TAGCCCAATCCCAATAGCAC
			Xetrov90017422_r3	GGAATAATGCAATTACAAAT
LOC116412144	LOC100493019_f1	CCCCTTTAAGATGTGCATGG	LOC100493019_r1	CGATGACGTTGGTTTCATGT
Xetrov90017432	Xetrov90017432_f1	ATTACATTGCCCGAATGAGC	Xetrov90017432_r1	GATCACCGGAGCAACACTCT
prkg1	trop_east_SNP4_f2	AGAGCTGAGCATAGAAAAGCAAG	trop_east_SNP4_r1	TCTCAAGTTGTAGCAGGCCAAG
TRINITY_DN3464_c0_g2_i2	Xt_DN3464_male_F2	CCCCACCCCTTACCCTGTAT	Xt_DN3464_male_R2	AGGCTGTGCTCCCTCAGATA

* LOC116412229 primers (Xetrov90017422_f1/r1) co-amplify multiple duplicated regions; for this reason, the f1/r3 primer pair were used in several individuals to amplify a male-specific allele, and confirm a sex linked SNP.

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