

## Supplementary materials for

### Genomic diversity and divergence in *Drosophila mauritiana*: multiple signatures of faster X evolution

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#### Methods

The Winters *sex-ratio* (SR) is one of three X-linked meiotic drive system in *Drosophila simulans* (Tao Araripe et al. 2007). This system is comprised of a compound drive locus: *Distorter on the X (Dox)* and *Mother of Dox (MDox)* and an autosomal suppressor, *Not much yang (Nmy)*. *Dox* originated as a duplicate copy of *MDox* and *Nmy* is a retrotransposed copy of *Dox* (Tao, Araripe et al. 2007). A functional copy of *Nmy* exists in both *D. simulans* and *D. mauritiana*, and is capable of suppressing drive, indicating that the Winters SR evolved in the *D. simulans* clade ancestor. These genes were previously shown to bear a signature of a selective sweep in *D. simulans* (Kingan et al. 2010). Here we characterize polymorphism at *Dox* and *MDox* in a larger sample of isofemale *D. mauritiana* lines (see main paper methods for collection details) to ask whether the Winters driver may be the target of selection.

Genomic DNA was extracted from single males using a modified protocol of the Wizard Genomic DNA Purification Kit from Promega. Polymerase chain reaction was performed using

Takara LA Taq polymerase following the manufacturer's instructions with a 5 minute extension time. PCR products were cleaned prior to sequencing using exoSAPit (USB). Previously published PCR primers for the *Dox* and *MDox* regions were used, which amplified complete genes as well as flanking sequence (Tao, Araripe, et al. 2007; Tao, Masly, et al. 2007; Kingan, et al. 2010; see supplementary table S14). Internal sequencing primers were designed using Primer3Plus (Untergasser, et al. 2007) and Amplify (Engels 2005); sequences are available upon request. Sequencing was performed on an ABI3730 capillary sequencer according to manufacturer's protocols; sequences were edited using Sequencher v.4.8 (Gene Codes Corp.). Many of the haplotypes from the *Dox* region have a series of identical or nearly identical copies of a 359-bp satellite DNA repeat element (Tao, Araripe, et al. 2007), which is related to the satellite block, *Zhr* (Ferree and Barbash 2009), which complicated the assembly of contigs. We digested the amplified PCR fragments with BslI, and compared the restriction fragment profile with that predicted from our contig to ensure the correct number of repeat elements were included in our alignments. 2× sequencing coverage was obtained for all samples, forward and reverse reads when possible, but in many cases, the lack of unique priming sites due to the tandemly arrayed repeat elements only allowed for 2× coverage in the same direction.

Alignments of sequences for each gene region, as well as between homologous regions of *Dox* and *MDox* were executed by eye using annotated sequence elements as “anchors” for the alignment. In some cases, the bl2seq program of BLAST was used for pair wise alignments (Tatusova and Madden 1999). To insure a proper alignment of each region, we performed a phylogenetic analysis of the 359-bp satellite elements for each gene to assign homology among repeats. For each locus, we extracted the repeat sequences from each sampled isofemale line and aligned the repeats by eye.

## Results

At the *MDox* region, we sample 26 chromosomes and compile a 5,526-bp alignment of the sequences (**supplementary table S16**). We infer that *MDox* is fixed in *D. mauritiana* because all 26 samples contain the gene insertion. We observe copy number variation in the 359-bp satellite elements that flank the *MDox* gene: copy number ranges from one to four tandemly arrayed repeats with most samples having two elements (**supplementary fig. S3**). **Fig. S3b** shows the neighbor-joining tree that was used to create an accurate alignment of the sequenced region (we obtained a similar trees using parsimony, not shown). In addition to the *D. mauritiana* lines sampled in this study, we include 69 previously sequenced *D. simulans* samples (Kingan, et al. 2010). We observe four clusters of repeat sequences with more than 75% bootstrap support and define these as *MDox* repeat types one through four.

At the *Dox* region, we sample the same 26 *D. mauritiana* chromosomes and compile an alignment of 8,503-bp. The ancestral *Dox*[*null*] allele, which lacks the *Dox* gene insertion, is more common than the derived allele, and is found in 20 of the sampled chromosomes (~77%) (Kingan, et al. 2010). One of the sampled strains (*R10*) has a ~3.2-kb deletion that is part of the *Dox* gene. Furthermore, we observe extensive copy number variation in the 359-bp satellite in *D. mauritiana*, with the number of elements ranging from four to seven (**supplementary fig. S2**). **Supplementary fig. S2b** also shows the neighbor-joining tree that was used to assemble an accurate alignment of the *Dox* region for our 26 *D. mauritiana* samples, as well as our previously collected *D. simulans* samples (Kingan, et al. 2010). We observe five clusters of elements with more than 56% bootstrap support and define these as *Dox* repeat types one through five. (No quantitative inferences are made about the evolution of repeat types from these

trees— they are simply used to compile the best alignment of the sequence data by clustering the most closely related repeat elements.)

## Literature Cited

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**Table S1**

Statistics describing the *de novo* assembly of the *mau12* genome.

measure	value
assembly size	124,206,178
number of contigs (> 1000-bp)	2,759
largest contig	2,989,439
N50	542,067
N75	235,193

**Table S2**

The number of contigs used to construct the syntenic pseudo-chromosomes. The length of the pseudo-chromosomes does not include any ambiguous bases that were used to fill gaps.

chromosome	contigs	length (bp)
<i>2L</i>	123	21,521,355
<i>2R</i>	151	18,987,217
<i>3L</i>	120	21,693,048
<i>3R</i>	59	25,652,786
<i>X</i>	150	17,830,043
total	603	105,684,449

**Table S3**

Details of the libraries sequenced for each strain of *Drosophila*. The penultimate column lists the number of reads mapping to the *mau12* reference genome after quality filtering.

strain	species	length (bp)	insert (bp)	mapped reads (x10 <sup>6</sup> )	accession
<i>mau12</i>	<i>mauritiana</i>	86	200	37.311	SRR483621
	<i>mauritiana</i>	100	3000	122.031	n/a
<i>R23</i>	<i>mauritiana</i>	76	350	57.315	n/a
	<i>mauritiana</i>	76	350	63.366	n/a
<i>R31</i>	<i>mauritiana</i>	76	350	47.173	n/a
	<i>mauritiana</i>	76	350	55.987	n/a
<i>R32</i>	<i>mauritiana</i>	76	350	58.338	n/a
	<i>mauritiana</i>	76	350	66.923	n/a
<i>R39</i>	<i>mauritiana</i>	76	350	55.664	n/a
	<i>mauritiana</i>	76	350	67.235	n/a
<i>R41</i>	<i>mauritiana</i>	76	350	60.61	n/a
	<i>mauritiana</i>	76	350	64.477	n/a
	<i>mauritiana</i>	76	350	61.971	n/a
<i>R44</i>	<i>mauritiana</i>	76	350	61.604	n/a
	<i>mauritiana</i>	76	350	67.161	n/a
<i>R56</i>	<i>mauritiana</i>	76	350	58.934	n/a
	<i>mauritiana</i>	76	350	68.625	n/a
<i>R61</i>	<i>mauritiana</i>	76	350	60.55	n/a
	<i>mauritiana</i>	76	350	62.871	n/a
	<i>mauritiana</i>	76	350	59.72	n/a
<i>R8</i>	<i>mauritiana</i>	76	350	61.991	n/a
	<i>mauritiana</i>	76	350	54.09	n/a
MD063	<i>simulans</i>	76	350	51.55	SRR1200800
	<i>simulans</i>	76	350	48.003	n/a
<i>w</i> <sup>501</sup>	<i>simulans</i>	100	500	37.974	SRR520334
<i>y; cn bw; sp</i>	<i>melanogaster</i>	146	300	46.061	SRR306626
	<i>melanogaster</i>	146	300	57.431	SRR306627
	<i>melanogaster</i>	146	300	60.617	SRR306628

**Table S4**

The 13 strains of *Drosophila* used in this study. Also included are the median read depth for each aligned position and the percent of *mau12* reference genome covered in each of the ten lines of *D. mauritiana*, two lines of *D. simulans*, and the reference strain of *D. melanogaster*, after quality filtering.

strain	species	depth	% covered
<i>mau12</i>	<i>mauritiana</i>	125	99.48
<i>R23</i>	<i>mauritiana</i>	84	98.83
<i>R31</i>	<i>mauritiana</i>	72	98.86
<i>R32</i>	<i>mauritiana</i>	87	98.84
<i>R39</i>	<i>mauritiana</i>	86	98.84
<i>R41</i>	<i>mauritiana</i>	130	98.86
<i>R44</i>	<i>mauritiana</i>	89	98.80
<i>R56</i>	<i>mauritiana</i>	90	98.86
<i>R61</i>	<i>mauritiana</i>	130	98.78
<i>R8</i>	<i>mauritiana</i>	81	98.86
MD063	<i>simulans</i>	70	98.18
<i>w</i> <sup>501</sup>	<i>simulans</i>	34	97.91
<i>y; cn bw; sp</i>	<i>melanogaster</i>	110	94.13



**Table S5**

Comparison of counts of genes on the X chromosome (X) and autosomes (A) with significant McDonald-Kreitman tests. Positive (pos.) and negative (neg.) gene categories are genes that show significant departure from neutral expectations in the direction of positive selection (excess nonsynonymous divergence) or negative selection (reduced nonsynonymous divergence), respectively, at the indicated significance cut-off ( $P < 0.05$  or  $P < 0.01$ ). Fisher's Exact Test (FET)  $P$ -value compares X-linked and autosomal counts for significant genes versus all genes tested.

test	gene category	X	A	FET $P$
polarized	pos ( $P < 0.05$ )	26	65	0.0011
	neg ( $P < 0.05$ )	4	22	1.0000
polarized	pos ( $P < 0.01$ )	11	12	0.0002
	neg ( $P < 0.01$ )	0	5	1.0000
	All tested	1,690	9,349	
unpolarized	pos ( $P < 0.05$ )	60	357	0.5359
	neg ( $P < 0.05$ )	45	222	0.5501
unpolarized	pos ( $P < 0.01$ )	33	111	0.0200
	neg ( $P < 0.01$ )	14	75	1.0000
	all tested	1,757	9,523	

**Table S6**

Top Gene Ontology (GO) process, function, and component terms enriched among genes with evidence of recurrent adaptive evolution in the history of the *D. mauritiana* and *D. melanogaster* lineages (unpolarized MK test).

GO category	description
Process	
GO:0044702	single organism reproductive process
GO:0022414	reproductive process
GO:0048610	cellular process involved in reproduction
GO:0044767	single-organism developmental process
GO:0032502	developmental process
GO:0007276	gamete generation
GO:0022412	cellular process involved in reproduction in multicellular organism
GO:0032862	activation of Rho GTPase activity
GO:0032863	activation of Rac GTPase activity
GO:0007568	aging
Function	
GO:0003676	nucleic acid binding
GO:0045502	dynein binding
GO:0004534	5'-3' exoribonuclease activity
GO:0008574	plus-end-directed microtubule motor activity
GO:0003723	RNA binding
GO:0008270	zinc ion binding
GO:0003696	satellite DNA binding
GO:0080023	3R-hydroxyacyl-CoA dehydratase activity
GO:0008409	5'-3' exonuclease activity
Component	
GO:0044611	nuclear pore inner ring
GO:0038201	TOR complex
GO:0032991	macromolecular complex
GO:0044427	chromosomal part
GO:0044428	nuclear part
GO:0005737	cytoplasm
GO:0043228	non-membrane-bounded organelle
GO:0043232	intracellular non-membrane-bounded organelle

**Table S7**

Top Gene Ontology (GO) process, function, and component terms enriched among genes with histories of recurrent adaptive evolution in the *D. mauritiana* lineage (polarized MK test).

GO category	description
Process	
GO:0007276	gamete generation
GO:0000184	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay
GO:0016071	mRNA metabolic process
GO:0030727	germarium-derived female germ-line cyst formation
GO:0048135	female germ-line cyst formation
GO:0016070	RNA metabolic process
GO:0000956	nuclear-transcribed mRNA catabolic process
GO:0010629	negative regulation of gene expression
GO:0006402	mRNA catabolic process
GO:0000291	nuclear-transcribed mRNA catabolic process, exonucleolytic
Function	
GO:0009055	electron carrier activity
GO:0003696	satellite DNA binding
GO:0017056	structural constituent of nuclear pore
GO:0004532	exoribonuclease activity
GO:0016896	exoribonuclease activity, producing 5'-phosphomonoesters
Component	
GO:0005700	polytene chromosome
GO:0043228	non-membrane-bounded organelle
GO:0043232	intracellular non-membrane-bounded organelle
GO:0005604	basement membrane
GO:0000151	ubiquitin ligase complex
GO:0044420	extracellular matrix part
GO:0031461	cullin-RING ubiquitin ligase complex
GO:0005694	chromosome
GO:0044428	nuclear part
GO:0044427	chromosomal part
GO:0044451	nucleoplasm part
GO:0044611	nuclear pore inner ring

**Table S8**

Mean and median values of *D. mauritiana* nucleotide diversity ( $\pi$ ) calculated for the 152 10-kb windows from SweepFinder with  $P < 0.05$  and 152 randomly drawn windows sorted by chromosome.  $P$ -values are the probability that SweepFinder windows have lower  $\pi$  based on 1000 bootstrap resampling replicates.

chrom	n windows	SweepFinder windows		random windows			$P$ -value
		mean	median	mean	0.025 CI	0.975 CI	
<i>2L</i>	56	0.0051	0.0028	0.0143	0.0123	0.0201	< 0.001
<i>2R</i>	19	0.0071	0.0054	0.0127	0.0112	0.0147	< 0.001
<i>3L</i>	10	0.0068	0.0062	0.0139	0.0115	0.0162	< 0.001
<i>3R</i>	6	0.0060	0.0059	0.0125	0.0101	0.0152	< 0.001
<i>X</i>	61	0.0023	0.0018	0.0085	0.0077	0.0093	< 0.001
aut.	91	0.0057	0.0041	0.0138	0.0124	0.0174	< 0.001

**Table S9**

Mean and median values of sequence divergence from *D. melanogaster* (average pairwise Jukes-Cantor divergence) calculated for the 152 10-kb windows from SweepFinder with  $P < 0.05$  and 152 randomly drawn windows sorted by chromosome.  $P$ -values are the probability that SweepFinder windows have higher divergence based on 1000 bootstrap resampling replicates.

chrom	n windows	SweepFinder windows		random windows			$P$ -value
		mean	median	mean	0.025 CI	0.975 CI	
<i>2L</i>	56	0.0623	0.0617	0.0425	0.0406	0.0445	< 0.001
<i>2R</i>	19	0.0563	0.0564	0.0410	0.0378	0.0444	< 0.001
<i>3L</i>	10	0.0407	0.0413	0.0417	0.0371	0.0468	0.592
<i>3R</i>	6	0.0493	0.0465	0.0410	0.0358	0.0470	0.013
<i>X</i>	61	0.0535	0.0547	0.0457	0.0438	0.0477	< 0.001
aut.	91	0.0577	0.0566	0.0420	0.0405	0.0435	< 0.001

**Table S10**

Mean and median values of Tajima's  $D$  ( $TD$ ) for *D. mauritiana* calculated for the 152 10-kb windows from SweepFinder with  $P < 0.05$  and 152 randomly drawn windows sorted by chromosome.  $P$ -values are the probability that SweepFinder windows have lower  $TD$  based on 1000 bootstrap resampling replicates.

chrom	n windows	SweepFinder windows		random windows			$P$ -value
		mean	median	mean	0.025 CI	0.975 CI	
<i>2L</i>	56	-0.1811	-0.1079	-0.2887	-0.3500	-0.2298	0.005
<i>2R</i>	19	-0.2784	-0.3205	-0.4331	-0.5409	-0.3217	0.013
<i>3L</i>	10	-0.3823	-0.3602	-0.2211	-0.3803	-0.0460	0.952
<i>3R</i>	6	-0.6639	-0.6393	-0.3917	-0.5832	-0.1801	0.988
<i>X</i>	61	-0.4690	-0.5440	-0.5298	-0.5991	-0.4541	0.079
aut.	91	-0.2553	-0.2309	-0.3181	-0.3684	-0.2677	0.024

**Table S11**

Mean and median values of  $Z_{nS}$  (average pairwise  $r^2$ ) for *D. mauritiana* calculated for the 152 10-kb windows from SweepFinder with  $P < 0.05$  and 152 randomly drawn windows sorted by chromosome.  $P$ -values are the probability that SweepFinder windows have higher  $Z_{nS}$  based on 1000 bootstrap resampling replicates.

chrom	n windows	SweepFinder windows		random windows			$P$ -value
		mean	median	mean	0.025 CI	0.975 CI	
<i>2L</i>	56	0.1647	0.1490	0.1364	0.1321	0.1417	< 0.001
<i>2R</i>	19	0.1333	0.1324	0.1245	0.1200	0.1310	0.024
<i>3L</i>	10	0.1391	0.1408	0.1371	0.1292	0.1484	0.270
<i>3R</i>	6	0.1730	0.1343	0.1310	0.1210	0.1437	0.006
<i>X</i>	61	0.2113	0.1457	0.1283	0.1223	0.1393	< 0.001
aut.	91	0.1559	0.1419	0.1336	0.1302	0.1374	< 0.001

**Table S12**

Polymorphism in the four genes in the putative sweep region X:16800000-17100000. Four nonsynonymous mutations are present and only three of those have arisen exclusively in the *D. mauritiana* lineage (one in the *CG32553* gene and two in the *CG43133* gene).

gene	<i>wup-A</i>	<i>CG32553</i>		<i>CG43133</i>					<i>ari-1</i>		
codon	133	27	35	26	31	47	60	95	102	127	380
<i>mel</i>	GAT	GCA	AAG	TTG	TAT	GCC	TTA	ATC	ACT	TCA	AAC
<i>sim</i>	...	...	CG.	...	...	...	...	...	...	...	...
<i>R8</i>	..C	.T.	C..	C..	..C	..A	A..	..G	..A	..G	..T
<i>mau12</i>	..C	.T.	C..	C..	..C	..A	A..	..G	..A	..G	..T
<i>R23</i>	..C	.T.	C..	C..	..C	..A	A..	..G	..A	..G	..T
<i>R31</i>	...	.T.	C..	C..	..C	..A	A..	..G	..A	..G	..T
<i>R32</i>	..C	.T.	C..	C..	..C	..A	A..	..G	..A	..G	..T
<i>R39</i>	...	.T.	C..	C..	..C	..A	A..	..G	..A	..G	..T
<i>R41</i>	..C	.T.	C..	C..	..C	..A	A..	..G	..A	..G	..T
<i>R44</i>	..C	.T.	C..	C..	..C	..A	A..	..G	..A	..G	..T
<i>R56</i>	..C	.T.	C..	C..	..C	..A	A..	..G	..A	..G	..T
<i>R61</i>	..C	.T.	C..	C..	..C	..A	A..	..G	..A	..G	..T
Change	syn	non	non	syn	syn	syn	non	non	syn	syn	syn
Amino Acid	D	A→V	K→Q Q→R*	I	Y	A	L→I	I→M	T	S	N

\*First nonsynonymous substitution occurred in *simulans* clade ancestor, second substitution in the *D. simulans* lineage.



**Table S13**

Percent masked sequence by chromosome arm for seven different repeat classes.

repeat class	<i>2L</i>	<i>2R</i>	<i>3L</i>	<i>3R</i>	<i>X</i>
LINES	0.0061	0.0087	0.0061	0.0029	0.0047
LTR elements	0.0097	0.0104	0.0064	0.0017	0.0023
DNA transposons	0.0043	0.0037	0.0028	0.0015	0.0026
unclassified	0.0076	0.0079	0.0061	0.0039	0.0065
satellites	0.0005	0.0004	0.0003	0.0005	0.0052
simple repeats	0.0174	0.0211	0.0215	0.0230	0.0354
low complexity	0.0027	0.0028	0.0032	0.0031	0.0051

**Table S14**

Identity of satellite DNA repeats present in block X.1 (see **fig. 3** from main text).

satellite	count	mean divergence
Sim359	45	15%
SAR_DM	26	20%
SAR2_DM	14	20%
SAT-1_Dsim	1	20%

## Table S15

The sequences of PCR primers used to amplify the Winters *sex-ratio* genes in *D. mauritiana*.

name	sequence
CG32702_SR6_F1 ( <i>MDox</i> )	GTAACGATGTGTACGCGCTTTGAGGTG
CG32702_SR6_R1 ( <i>MDox</i> )	GGTCGCGGATTCACATTGCTCTATAACC
DoxF4	AAGCAGTTCCCAAAGAAAGAGAGCAGAGCAACT
DoxR4	CCAGCTCAAACCACAGGGAGACTGTACATA

**Table S16***Drosophila mauritiana* strains sequences in this study.

strain	<i>Dox</i> genotype	<i>MDox</i> genotype
<i>mau12</i> *	<i>Dox</i> [null]	<i>MDox</i>
<i>R2</i>	<i>Dox</i>	<i>MDox</i>
<i>R3</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R4</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R6</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R7</i>	<i>Dox</i>	<i>MDox</i>
<i>R8</i> *	<i>Dox</i> [null]	<i>MDox</i>
<i>R9</i>	<i>Dox</i>	<i>MDox</i>
<i>R10</i>	<i>Dox</i> [del]**	<i>MDox</i>
<i>R11</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R12</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R13</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R16</i>	<i>Dox</i>	<i>MDox</i>
<i>R17</i>	<i>Dox</i>	<i>MDox</i>
<i>R18</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R19</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R20</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R22</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R23</i> *	<i>Dox</i> [null]	<i>MDox</i>
<i>R26</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R27</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R30</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R31</i> *	<i>Dox</i> [null]	<i>MDox</i>
<i>R32</i> *	<i>Dox</i> [null]	<i>MDox</i>
<i>R35</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R38</i>	<i>Dox</i> [null]	<i>MDox</i>
<i>R39</i> *	<i>Dox</i> [null]	<i>MDox</i>

\*Present in the population genomic dataset.

\*\*See **fig. S3** and supplementary text.

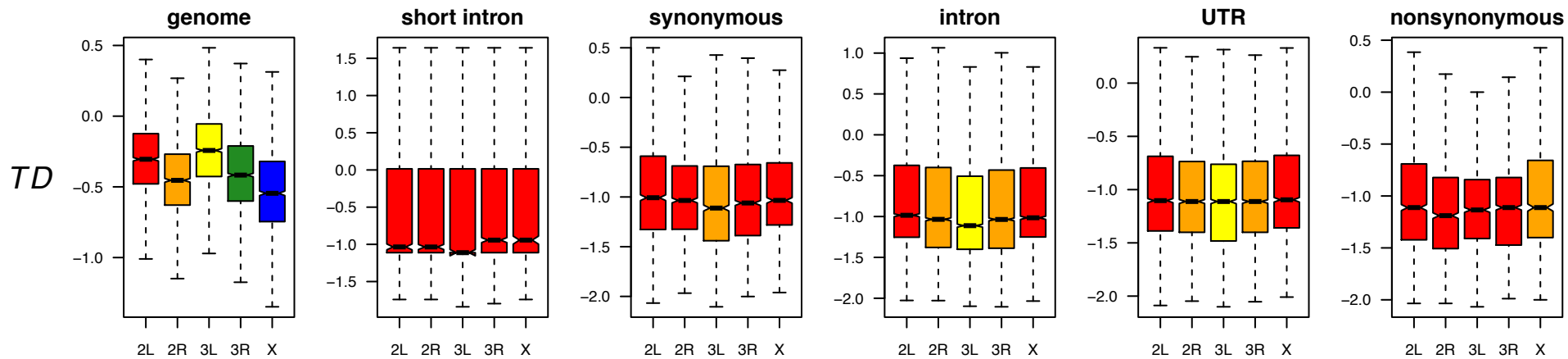
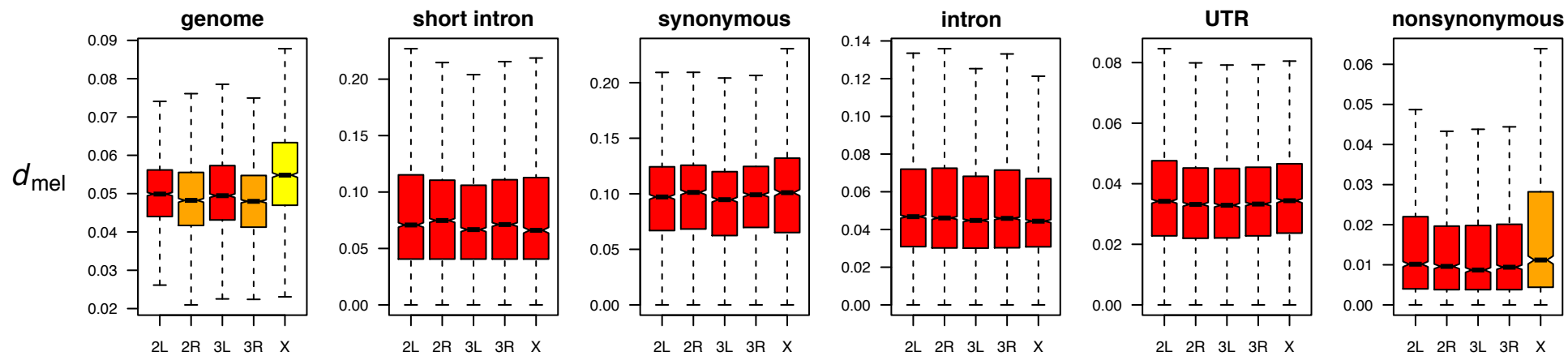
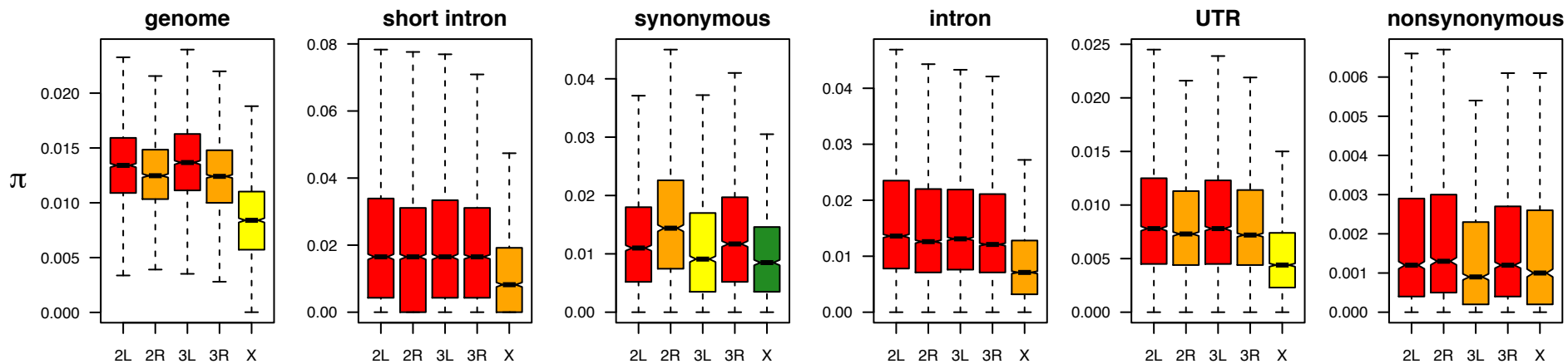
## Supplementary Figure Legends

**FIG. S1.**— Distribution of the average pairwise nucleotide distance ( $\pi$ ), the average Jukes-Cantor corrected distance from *D. melanogaster* ( $d_{\text{mel}}$ ), and the summary of the site frequency spectrum, Tajima's  $D$  ( $TD$ ), by chromosome for the whole genome (10-kb windows) and five different sequences classes. Box plots that are different colors have significantly different distributions (Mann-Whitney  $U$ ,  $P < 0.05$  after correcting for 10 pairwise comparisons).

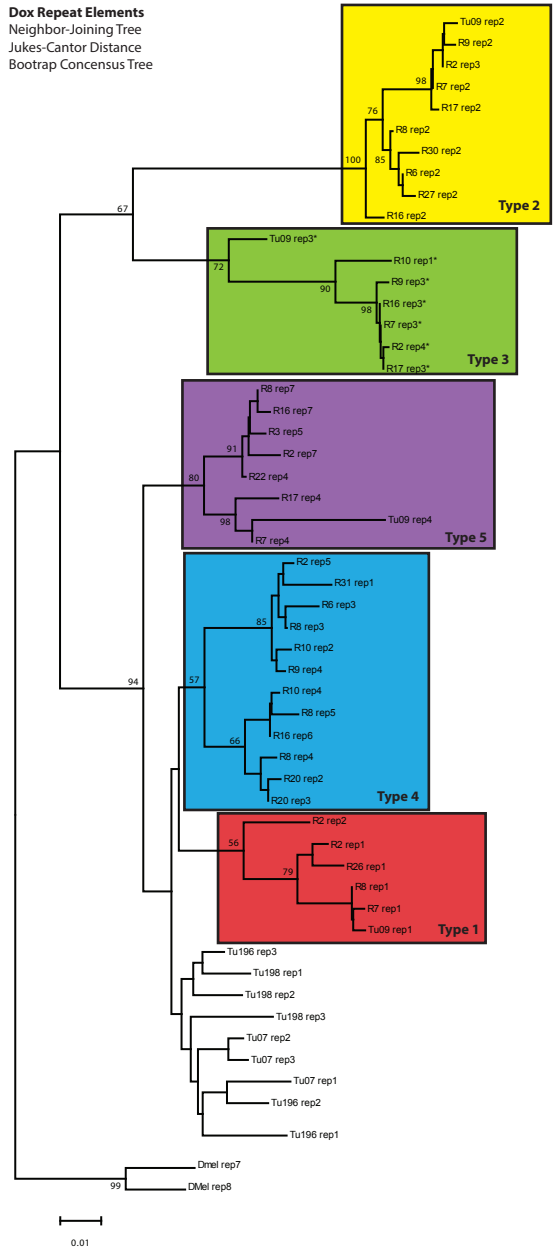
**FIG. S2.**— Illustration of the alignment of *Dox* locus for *D. mauritiana* (above) and *D. simulans* (below). The 359-bp satellite DNA repeats are shown as rectangles. The colors correspond to repeat types based on the phylogenetic analysis; grey repeat types are present in only *Dox[null]* haplotypes of *D. simulans* and do not form a distinct clade (Panel B). “Type Sample” refers to the identity of one of the isofemale lines with the haplotype shown. Frequency is the number of times the haplotype appears in the sample. Length is the total length of the sequenced haplotype, which included repeats and flanking sequence. The *Dox* insertion is shown as a black triangle and is not drawn to scale. Gaps introduced into the alignment are shown as dashed lines. B) Bootstrap consensus neighbor-joining tree based on Jukes-Cantor genetics distance. The tree is rooted with *D. melanogaster* repeat elements from the *Dox* locus. Numbers at nodes are bootstrap values (values not shown are less than 40). Colors indicate repeat types as in Figure 3.4. “Tu” samples are *D. simulans*, “R” samples are *D. mauritiana*. Repeat elements for each sample are numbered in order (e.g., “R8 rep 7” and “R3 rep 5” are homologous and are both type 5 shown as purple). Repeat elements that contain the *Dox* insertion were concatenated and are indicated with an asterisk.

**FIG. S3.**— Illustrative alignment of the *MDox* locus for *D. mauritiana* (above) and *D. simulans* (below). The 359-bp satellite DNA repeats are shown as rectangles. Colors correspond to repeat

types based on the phylogenetic analysis; grey repeat types are present in only *MDox[null]* haplotypes of *D. simulans* and do not form a distinct clade (Panel B). “Type Sample” refers to the identity of one of the isofemale lines with the haplotype shown. Frequency is the number of times the haplotype appears in the sample. Length is the total length of the sequenced haplotype. The *MDox* insertion is shown as a black triangle and is not drawn to scale. Gaps introduced into the alignment are shown as dashed lines. B) Bootstrap consensus neighbor-joining tree based on Jukes-Cantor genetics distance. The tree is rooted with *D. melanogaster* repeat elements from the *Dox* locus. Numbers at nodes are bootstrap values (values not shown are less than 40). Colors indicate repeat type as in Panel A. “Tu” samples are *D. simulans*, “R” samples are *D. mauritiana*. Repeat elements for each sample are numbered in order (e.g., “R18 rep 4” and “R6 rep 2” are homologous and are both type 4, shown as blue). Repeat elements that contain the *MDox* insertion were concatenated (i.e., the *MDox* insertion was excised) and are denoted with an asterisk.



Type	Sample	Frequency	Number of Repeats	Length (bp)	Type 1	Type 2	Type 3	Type 4	Type 5
<i>D. mauritiana</i>	R8	4	7	3830					
	R6	5	6	3471					
	R3	4	5	3112					
	R27	2	5	3141					
	R31	1	5	3162					
	R22	2	4	2753					
	R20	1	4	2801					
	R26	1	4	2405					
	R2	1	7	7027					
	R16	1	7	7166					
R9	1	5	6355						
R10	1	5	3778						
R7	2	4	5667						
<i>D. simulans</i>	Tu9	67	4	6062					
	Tu07	1	3	2410					
	Tu196	2	3	2397					
	Tu198	1	3	2410					





	Type Sample	Frequency	Number of Repeats	Length (bp)	Type 1	Type 2	Type 3	Type 4
<i>D. mauritiana</i>	R18	1	4	5260				
	R20	1	4	5152				
	R6	23	2	4544				
	R19	1	1	4179				
<i>D. simulans</i>	Tu9	65	2	4508				
	Tu07	1	3	3618				
	Tu184	1	3	3604				
	Tu196	1	2	3349				
	Tu197	1	1	2845				

**MDox Repeat Elements**  
Neighbor-Joining Tree  
Jukes-Cantor Distance  
Bootstrap Concensus Tree

