

D. melanogaster

Su(Ste)-specific 663 (663bp)

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ACCGGAGAATTC

cryme3 (386bp)

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D. simulans

F6.3 (471bp)

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CCAGCCGCCCCCTGGAGGTCCACCGGTAAGTAATTCTATGAATTTAGTCCT
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crysi3 (380bp)

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F4C2 (1041bp)

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20F1 (775 bp)

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17F7 (920 bp)

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F39A (3559 bp)

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D. sechellia

crysec3 (382bp)

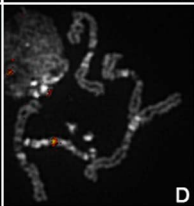
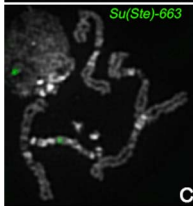
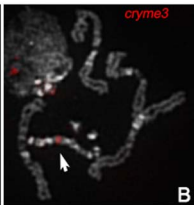
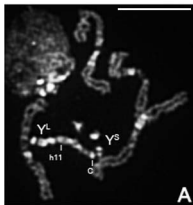
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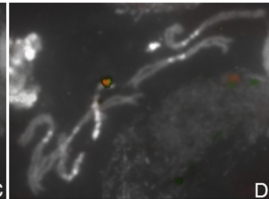
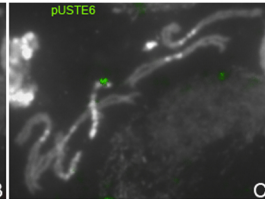
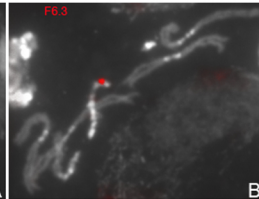
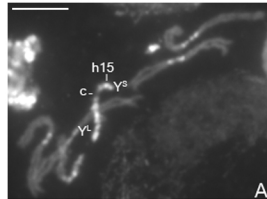
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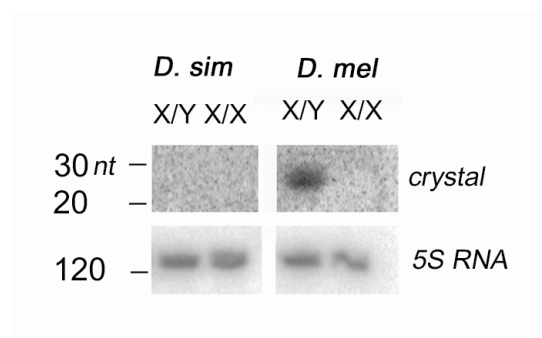
D. mauritiana

crymau3 (389 bp)

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Supplementary Materials:

Figure S1. Sequences of the clones obtained from the indicated *Drosophila* species.

Figure S2. FISH of the cryme3 and *Su(Ste)*-specific 663 probes on mitotic chromosomes of *D. melanogaster*.

Localization by fluorescence in *situ* hybridization of cryme3-Cy2 labeled (red)(B) and *Su(Ste)*-specific 663-FluorX labeled (green) (C), on mitotic chromosomes of *D. melanogaster*, stained with DAPI. Both probes map in the h11 region of Y chromosome; (D) a merge of the two hybridization patterns; the yellow signal on the merged figure indicates the co-localization of the two probes. Centromere (c). Scale bar indicates 5 μ m.

Figure S3. Multialignment of the F6.3 sequence, *Ste*, *her^{Ste}*, *Su(Ste)*, *PCKR* and the sequences of *D. melanogaster* genome obtained using the BLAT algorithm on the UCSC Genome Browser (<https://genome.ucsc.edu/cgi-bin/hgBlat> accessed on 28 October 2022).

Figure S4. FISH of the F6.3 and pUSTE6 probes on mitotic chromosomes of *D. simulans*.

Localization by fluorescence in *situ* hybridization of F6.3-Cy2 labeled (red)(B) and pUSTE6-FluorX labeled (green) (C), on mitotic chromosomes of *D. simulans*, stained with DAPI. Both signals are in the h15 region of the Y chromosome; (D) a merge of the two hybridization patterns; the yellow signal on the merged figure indicates the co-localization of the two probes. Only the strong hybridization signal is shown. Centromere (c). Scale bar indicates 5 μ m.

Figure S5. piRNA detection by Northern Blot analysis. Enriched small RNA extracts from testes and ovaries of the indicated genotypes, hybridized with the oligonucleotide named crystal. 5S RNA was used as a loading control.

Table S1. Sequence similarity between the F6.3 sequence and the orthologous sequences of *D. melanogaster*.

Ok.matrix.identity.F6.3_output_

Sequence	Identity vs F6.3 (%)
F6.3/1-471	100
dmel.X_Ste_1/1-711	71
dmel.Contig5_hetSte./1-699	71
dmel.Y_SuSte_scaffol/1-702	68
dmel.Y_PCKR_scaffold/1-539	70
chrUn_DS483780v1:334/258-593	76
chrUn_DS483780v1:362/314-664	75
chrY:1123325-1123659/1-335	79
chrUn_DS485490v1:316/1-333	78
chrY_DS483875v1_rand/1-297	69
chrX:14046769-140469/1-223	75
chrX:14048008-140482/1-223	75
chrX:14059431-140596/1-223	75
chrX:14049277-140494/1-223	75
chrX:14053084-140533/1-223	75
chrX:14055623-140558/1-223	75
chrX:14056892-140571/1-223	75
chrX:14050546-140507/1-223	74
chrX:14060699-140609/1-223	74
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chrY:1039612-1039767/1-156	80
chr2R:24499551-24499/1-151	75
chrUn_DS483780v1:348/1-123	71
chrUn_DS485490v1:699/1-141	75
chrY:866508-866586/1-79	84
chrY:872386-872466/1-81	84
chrUn_DS483780v1:394/1-170	82
chrY_CP007109v1_rand/1-187	62
chrY_CP007116v1_rand/1-83	74
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chrY:1042347-1042396/1-50	84
chrY:2549479-2549528/1-50	82
chrY:2574779-2574856/1-78	71
chrY:2586154-2586231/1-78	71
chrY:2591504-2591581/1-78	71
chrX:23352202-233522/1-38	94