

Table S1. Median read depth (*D*), total alignment length (*L*), and the percent of the *D. melanogaster* reference genome length covered by the alignment (*C*) for each of the three species of the *D. simulans* clade and for the entire four species alignment, following the filtering out of low quality short-read alignments.

Region	<i>D. simulans</i>			<i>D. mauritiana</i>			<i>D. sechellia</i>			Entire alignment	
	<i>D</i>	<i>L</i>	<i>C</i> (%)	<i>D</i>	<i>L</i>	<i>C</i> (%)	<i>D</i>	<i>L</i>	<i>C</i> (%)	<i>L</i>	<i>C</i> (%)
2L	17	19,107,171	83.03	20	17,549,685	76.26	45	19,663,519	85.45	16,687,022	72.52
2R	17	17,319,605	81.90	20	15,967,117	75.51	44	17,746,125	83.92	15,170,506	71.74
3L	17	20,192,246	82.27	20	18,535,219	75.52	44	20,797,612	84.74	17,624,525	71.81
3R	17	24,705,696	88.53	21	22,873,761	81.97	45	25,283,587	90.61	21,881,103	78.41
4	13	602,998	44.61	20	471,266	34.86	36	648,180	47.95	414,733	30.68
X	14	17,587,324	78.43	16	15,601,192	69.58	35	18,410,556	82.11	14,391,879	64.18
mt	1345	15,269	78.23	889	14,541	74.50	2906	15,850	81.21	14,389	73.73
Total	16	99,530,362	82.67	20	91,012,781	75.59	44	102,565,429	85.19	86,184,157	71.58

Table S2. Average frequency of optimal codons (F_{op}) across genes.

Region	sim	mau	sec
<i>2L</i>	0.530	0.530	0.528
<i>2R</i>	0.547	0.547	0.544
<i>3L</i>	0.533	0.534	0.530
<i>3R</i>	0.542	0.543	0.540
Autosomes	0.539	0.539	0.536
<i>X</i>	0.574	0.574	0.571
<i>4</i>	0.266	0.266	0.265
Total	0.543	0.543	0.540

Table S3. The number of coding substitutions between the *D. simulans*-clade ancestor and each extant species.

Species	Chromosome	Nonsynonymous	Preferred	Unpreferred	Equivalent	Total Codons
sim	2R	3551.25	4646.67	8211.50	1702.58	101906
mau	2R	3597.50	4470.00	8467.00	1724.50	101700
sec	2R	5452.83	4225.67	10703.67	1874.83	102254
sim	2L	3169.50	4661.50	7835.00	1637.00	94610
mau	2L	3380.00	4572.50	7918.50	1576.00	94304
sec	2L	4816.00	4281.50	9511.17	1739.33	94851
sim	3R	4395.50	5476.67	9933.50	2119.33	122597
mau	3R	4374.33	5291.83	10059.50	2064.33	122312
sec	3R	6495.75	5016.50	12687.75	2205.00	122917
sim	3L	3255.50	4465.50	7615.67	1622.33	92868
mau	3L	3465.33	4484.50	7699.17	1680.00	92658
sec	3L	5148.83	4099.50	9458.17	1830.50	93148
sim	X	2128.00	2522.00	3354.50	644.50	61754
mau	X	2110.00	2202.50	3420.50	578.00	61645
sec	X	3064.00	1875.50	5055.50	683.00	62040
sim	4	63.00	55.00	63.00	37.00	2760
mau	4	85.00	41.00	56.00	33.00	2759
sec	4	112.00	38.00	63.00	31.00	2760