

Supplementary Materials

S1. The distribution of reads and informative markers among individuals.

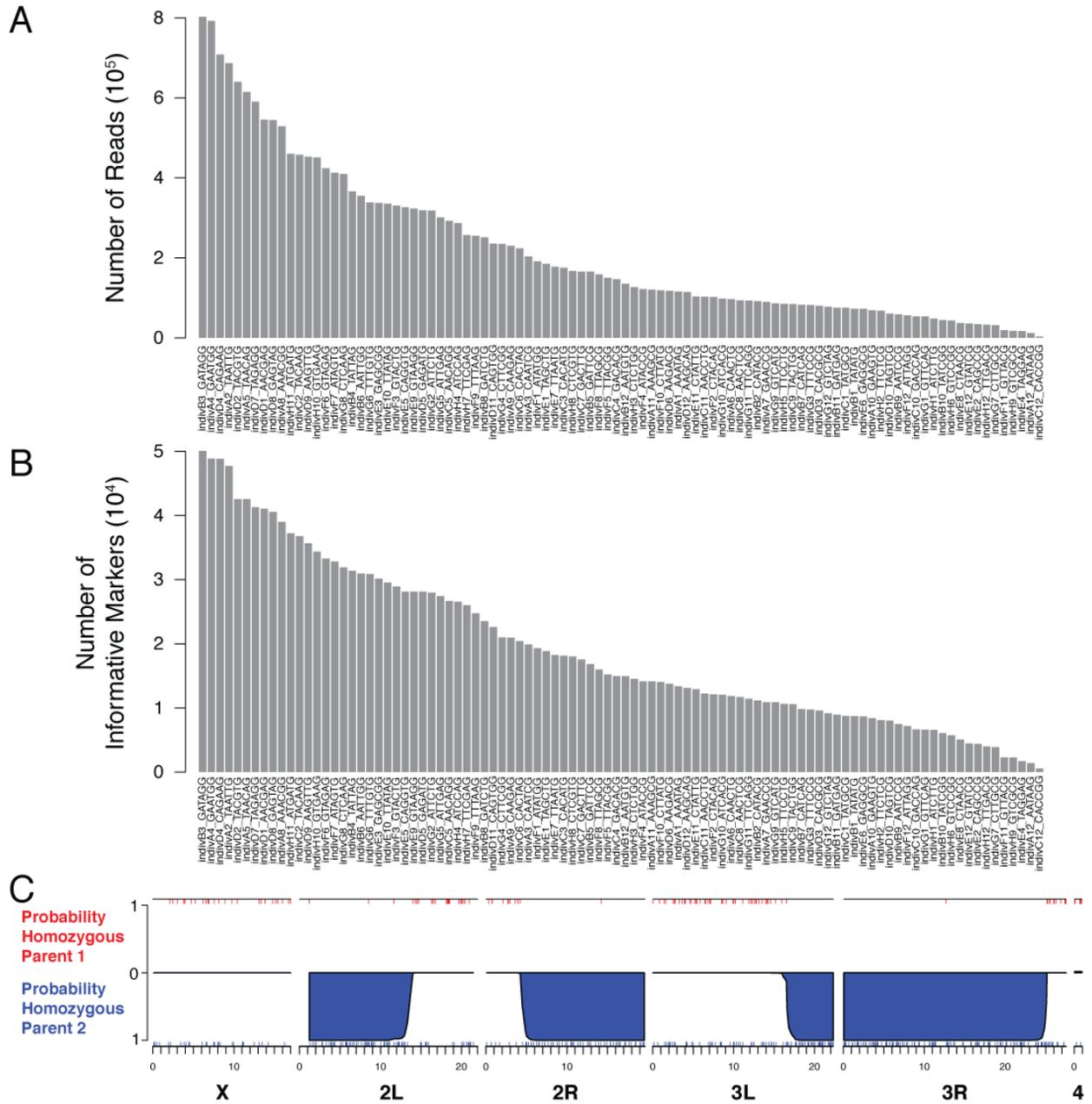


Figure S1. The distribution of reads (A) and informative markers (B) among 96 individuals in the F1-backcross experiment. Panel C shows the ancestry plot (analogous to Figure 2 in the main text) for the least-covered individual (C12_CACCGG).

S2. The effect of varying the genotyping error parameter γ on the resolution of recombination breakpoints.

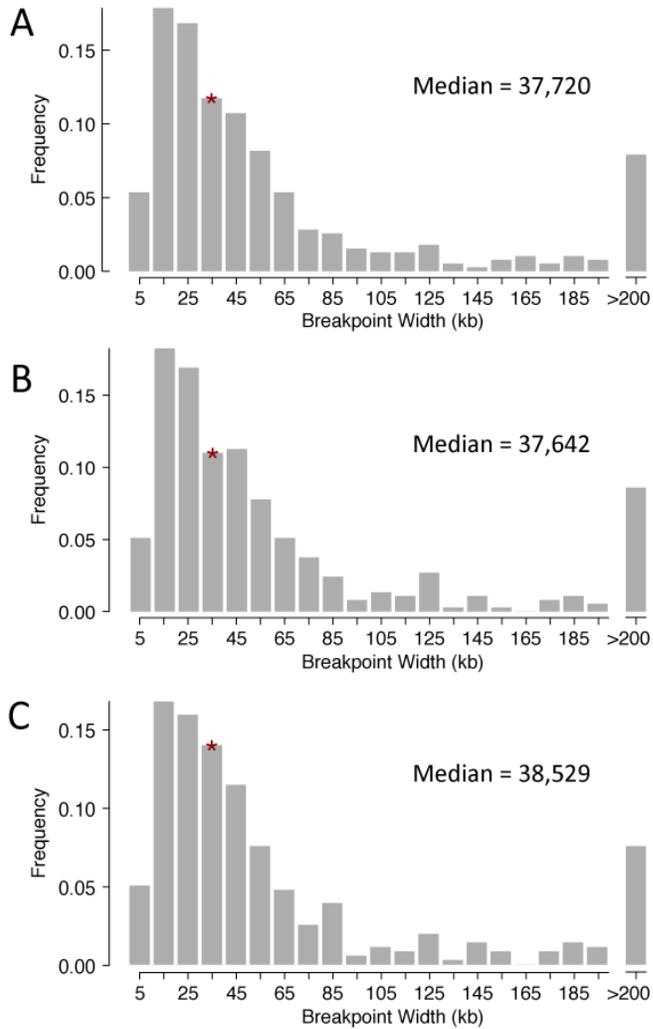


Figure S2. Histograms of estimated breakpoint widths in the *D. simulans/D. sechellia* F1-backcross experiment. (A) $\gamma = 0.005$, 465 breakpoints. (B) $\gamma = 0.01$, 441 breakpoints. (C) $\gamma = 0.02$, 419 breakpoints. Asterisks denote the median breakpoint width. Breakpoints that were >200kb can all be attributed to the 4 breakpoints identified in the least covered individual in this experiment (Figure S1C).

S3. Mapping unassembled contigs of the *Drosophila simulans* genome assembly.

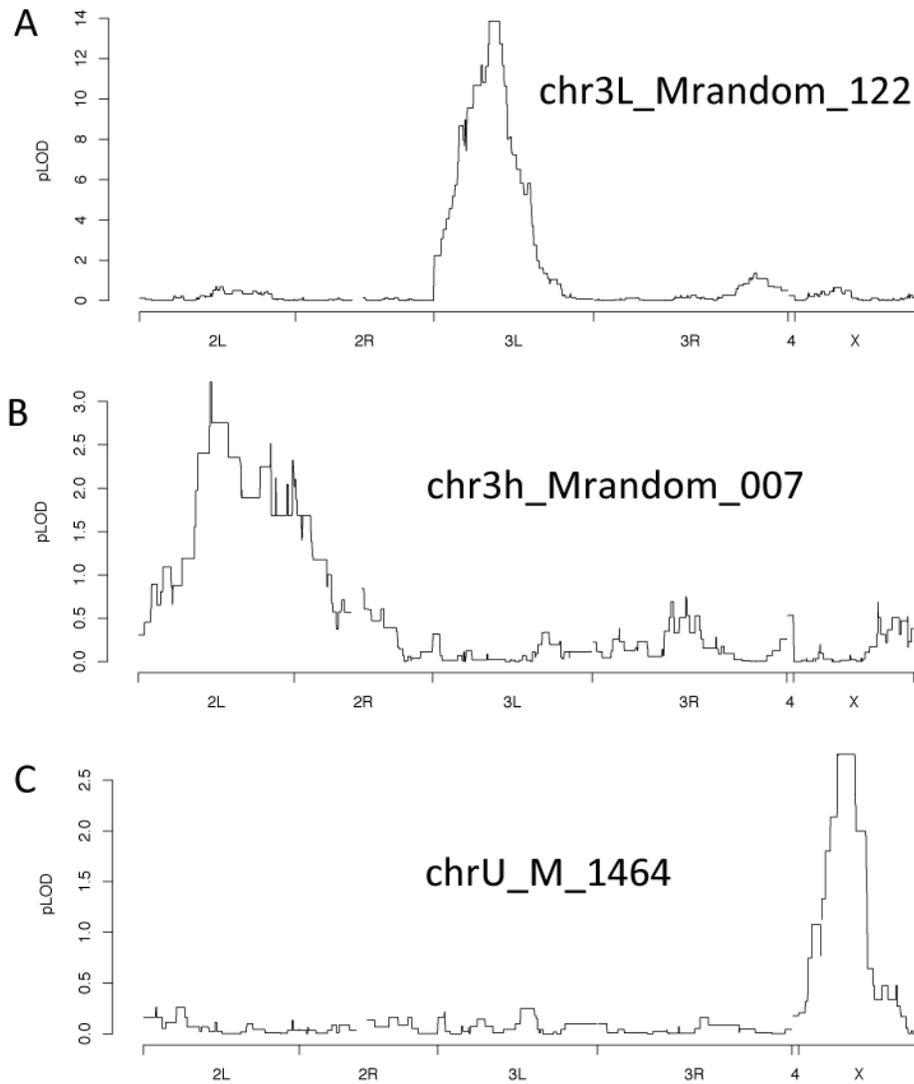


Figure S3. Examples of mapping unassembled contigs. All figures use the mean ancestry call for a given unassembled contig. Shown are examples of (A) confirming an assignment based on sequence homology, (B) refuting an assignment based on sequence homology and (C) assigning an unassembled contig that lacks an assignment based on sequence homology. See attached table of linkage assignments for 404 unassembled contigs of the *D. simulans* genome assembly in “Supplementary Table 3”.

Linkage assignments for 404 unassembled contigs of the D. simulans genome assembly

Legend

MaxLODMean The maxLOD based on the mean ancestry call for the contig

n Number of individuals with ancestry calls

MaxPossLOD Maximum possible LOD with complete linkage

MaxLODMeanContig Contig with max LOD peak

MaxLODMeanPos Position of max LOD peak

ContigLength Length of contig in base pairs

contig	MaxLODMean	n	MaxPossLOD	MaxLODMeanContig	MaxLODMeanPos	ContigLength
chr2h_Mrandom_002	3.887569314	82	24.68445964	2L	10237907	40401
chr2h_Mrandom_003	13.48427674	94	28.29681959	2R	2369687 218562	
chr2h_Mrandom_004	2.037636177	92	27.6947596	2L	8471560 91897	
chr2h_Mrandom_005	13.08445762	94	28.29681959	3R	153471 306220	
chr2h_Mrandom_008	12.90542587	84	25.28651964	3L	19857777	29912
chr2h_Mrandom_009	20.52767484	95	28.59784959	2L	21677421	179334
chr2h_Mrandom_012	9.165616182	85	25.58754963	2L	20983209	52392
chr2h_Mrandom_013	6.767024916	93	27.9957896	2R	46838 196181	
chr2h_Mrandom_014	2.128066711	89	26.79166961	2L	10283145	71639
chr2h_Mrandom_015	6.102780499	94	28.29681959	2L	19463431	149851
chr2h_Mrandom_017	3.39534775	94	28.29681959	3R	19195297	109391
chr2h_Mrandom_018	17.3279919	91	27.39372961	2R	194121 43741	
chr2h_Mrandom_019	2.393463867	81	24.38342965	2L	10241780	42924
chr2h_Mrandom_021	7.197628002	95	28.59784959	2R	1319940 73380	
chr2h_Mrandom_022	17.78577659	96	28.89887958	2L	21267355	316740
chr2h_Mrandom_024	12.26733124	95	28.59784959	3R	212742 216363	
chr2h_Mrandom_028	2.834825828	72	21.67415969	2L	21820219	13073
chr2h_Mrandom_036	5.317990611	55	16.55664976	2R	1011474 24982	
chr2h_Mrandom_037	2.283431458	22	6.622659905	2L	11484133	8465
chr2h_Mrandom_038	3.143383808	93	27.9957896	2L	11523213	304777
chr2h_Mrandom_054	6.089602058	72	21.67415969	3L	19472751	26151
chr2h_Mrandom_058	3.371071988	90	27.09269961	2L	21267355	86047
chr2h_Mrandom_060	24.4093502	92	27.6947596	2R	677931 86170	
chr2h_Mrandom_061	18.02946936	88	26.49063962	2L	19595913	40959
chr2h_Mrandom_062	8.875321111	83	24.98548964	2L	20441103	25800
chr2L_Mrandom_005	3.635905079	26	7.826779887	2L	726393 1514	
chr2L_Mrandom_027	9.805654556	72	21.67415969	2L	722179 17818	
chr2L_Mrandom_069	3.099999253	29	8.729869874	2L	3921054 5110	
chr2L_Mrandom_070	2.690453665	12	3.612359948	2L	7299319 1776	
chr2L_Mrandom_074	17.26146548	81	24.38342965	2L	5058318 38155	
chr2L_Mrandom_079	4.484481943	28	8.428839879	2L	5647688 2214	
chr2L_Mrandom_082	3.133584381	26	7.826779887	2L	8022567 1501	
chr2L_Mrandom_141	5.4132839	49	14.75046979	2L	14144161	5353
chr2L_Mrandom_145	5.25141443	54	16.25561977	2L	13698038	6166
chr2L_Mrandom_146	7.656194603	59	17.76076974	2L	13624613	14018
chr2L_Mrandom_170	2.299149462	14	4.214419939	3R	13323456	9514
chr2L_Mrandom_180	2.597102315	20	6.020599913	2L	18161102	2102
chr2L_Mrandom_188	10.29468355	62	18.66385973	2L	17867068	28328
chr2L_Mrandom_201	4.768974638	52	15.65355977	2L	18491908	2446
chr2L_Mrandom_216	8.083757324	80	24.08239965	2L	19595887	33594
chr2L_Mrandom_222	2.593072874	22	6.622659905	2L	20735272	1138
chr2L_Mrandom_224	6.327693851	51	15.35252978	2R	1640200 12471	
chr2L_Mrandom_226	16.31328714	77	23.17930967	2L	20983209	18821
chr2R_Mrandom_005	4.107687697	52	15.65355977	2R	31878 12450	
chr2R_Mrandom_007	3.884082152	74	22.27621968	2L	18757275	23562
chr2R_Mrandom_009	11.28557586	92	27.6947596	2L	21616760	132772
chr2R_Mrandom_011	2.090135044	93	27.9957896	2L	13698038	44629
chr2R_Mrandom_018	25.05776	92	27.6947596	2L	20532634	102699
chr2R_Mrandom_027	2.254489591	17	5.117509926	2L	14619942	15412
chr2R_Mrandom_069	3.844634026	27	8.127809883	2R	2504383 7354	
chr2R_Mrandom_070	3.89121565	51	15.35252978	2R	902780 5106	
chr2R_Mrandom_079	7.858225805	60	18.06179974	2R	194121 12736	
chr2R_Mrandom_154	3.355070464	34	10.23501985	2R	4566675 3351	
chr2R_Mrandom_164	2.113796045	32	9.632959861	2L	12847455	9067
chr2R_Mrandom_206	16.48028143	81	24.38342965	2R	2225138 52908	
chr2R_Mrandom_210	2.8290238	14	4.214419939	2L	17662726	2264
chr2R_Mrandom_218	2.027168366	25	7.525749892	2L	20735272	11488
chr2R_Mrandom_225	5.308429575	34	10.23501985	2L	19735924	4287
chr2R_Mrandom_250	2.100651111	10	3.010299957	2L	12552508	10990
chr2R_Mrandom_274	2.971463629	27	8.127809883	2R	2724463 7291	

chr2R_Mrandom_280	2.208270495	28	8.428839879	2L	21150839	1302
chr2R_Mrandom_332	3.596760362	26	7.826779887	2L	19200844	3558
chr2R_Mrandom_348	8.474547985	58	17.45973975	2R	3734088	12746
chr2R_Mrandom_352	2.977664887	32	9.632959861	2R	5918033	1624
chr2R_Mrandom_357	2.303783659	22	6.622659905	2R	2504383	2208
chr2R_Mrandom_363	2.350954708	27	8.127809883	2R	5226456	13653
chr2R_Mrandom_388	5.8454928	45	13.5463498	2R	6310188	13222
chr2R_Mrandom_399	5.604984728	44	13.24531981	2R	5578661	7559
chr2R_Mrandom_420	13.973018	78	23.48033966	2R	7254131	36738
chr2R_Mrandom_448	2.758432978	24	7.224719896	2R	6326659	2079
chr2R_Mrandom_471	2.661883266	22	6.622659905	2R	7941959	5703
chr2R_Mrandom_521	5.765871303	65	19.56694972	2R	9951425	7254
chr2R_Mrandom_524	2.116798802	36	10.83707984	2R	11557650	7108
chr2R_Mrandom_529	4.000730575	50	15.05149978	2R	10059432	6478
chr2R_Mrandom_548	3.22849866	42	12.64325982	2R	11553934	9395
chr2R_Mrandom_562	3.400278827	51	15.35252978	2R	13710779	7067
chr2R_Mrandom_568	2.934644178	35	10.53604985	2R	9837546	8669
chr2R_Mrandom_569	5.376359288	46	13.8473798	2R	13740553	3629
chr2R_Mrandom_571	2.804583201	27	8.127809883	2R	12662824	5667
chr2R_Mrandom_574	2.100009332	16	4.816479931	2R	14365195	1928
chr2R_Mrandom_612	2.195671513	37	11.13810984	2R	19543458	14018
chr2R_Mrandom_618	2.607398801	27	8.127809883	2R	15770833	17615
chr2R_Mrandom_639	9.357185526	64	19.26591972	2R	19067766	10981
chr2R_Mrandom_643	2.628255992	29	8.729869874	2R	19518287	6425
chr3h_Mrandom_001	22.17565888	92	27.6947596	3R	253789	115719
chr3h_Mrandom_002	5.705412953	94	28.29681959	2L	21822045	115439
chr3h_Mrandom_003	3.367394178	68	20.47003971	3R	1402855	63371
chr3h_Mrandom_007	3.224270159	46	13.8473798	2L	10288016	60118
chr3h_Mrandom_009	2.000394028	13	3.913389944	3R	568129	13231
chr3h_Mrandom_012	7.171526024	47	14.1484098	3R	153471	40392
chr3h_Mrandom_013	24.57668847	95	28.59784959	3L	20601042	187459
chr3h_Mrandom_015	10.09506216	72	21.67415969	3R	220594	22992
chr3h_Mrandom_016	19.31957302	89	26.79166961	2L	21472331	35553
chr3h_Mrandom_018	17.23784515	88	26.49063962	2R	215498	51980
chr3h_Mrandom_028	13.07469749	83	24.98548964	3L	19472751	31405
chr3h_Mrandom_029	3.632286521	93	27.9957896	3R	153471	109586
chr3h_Mrandom_030	9.337531734	76	22.87827967	3L	19336088	26586
chr3h_Mrandom_032	7.140579187	82	24.68445964	3R	153471	26599
chr3h_Mrandom_033	15.5397602	82	24.68445964	3L	19857777	25518
chr3h_Mrandom_037	17.95360664	89	26.79166961	2R	194121	86402
chr3h_Mrandom_049	2.251636359	84	25.28651964	2L	10283145	31089
chr3h_Mrandom_051	6.606254408	90	27.09269961	3R	243304	59267
chr3h_Mrandom_054	4.041054429	21	6.321629909	2R	28893	18422
chr3L_Mrandom_003	2.843378872	49	14.75046979	2L	10234933	9745
chr3L_Mrandom_004	7.518609659	57	17.15870975	3L	359788	8464
chr3L_Mrandom_009	2.162416068	18	5.418539922	3L	3084729	1145
chr3L_Mrandom_021	6.798018817	57	17.15870975	3L	1452497	29569
chr3L_Mrandom_041	2.298124845	16	4.816479931	3L	4265403	1487
chr3L_Mrandom_050	11.89962616	79	23.78136966	3L	3506536	16808
chr3L_Mrandom_056	3.010891352	29	8.729869874	3L	9334817	2700
chr3L_Mrandom_069	3.071572646	27	8.127809883	3L	4618023	2242
chr3L_Mrandom_070	2.170192147	26	7.826779887	3L	5117608	1495
chr3L_Mrandom_072	2.118097958	15	4.515449935	3L	4984075	6770
chr3L_Mrandom_077	12.06980575	67	20.16900971	3L	5144677	14027
chr3L_Mrandom_078	4.335800563	43	12.94428981	3L	4530250	2449
chr3L_Mrandom_096	4.695232228	46	13.8473798	3L	7592306	9785
chr3L_Mrandom_099	3.250802265	34	10.23501985	3L	7589046	4718
chr3L_Mrandom_100	4.283818984	48	14.44943979	3L	8760209	1671
chr3L_Mrandom_120	2.615961318	26	7.826779887	3L	7592306	9173
chr3L_Mrandom_121	4.849778195	43	12.94428981	3L	9233903	6408
chr3L_Mrandom_122	13.86859466	83	24.98548964	3L	8937239	9173
chr3L_Mrandom_141	2.177042845	18	5.418539922	3L	10728196	5557
chr3L_Mrandom_172	3.48030663	33	9.933989857	3L	12373829	1546
chr3L_Mrandom_178	3.686549962	81	24.38342965	X	15354273	39871
chr3L_Mrandom_179	4.754480631	51	15.35252978	3L	12772641	5422
chr3L_Mrandom_183	2.345719736	27	8.127809883	3L	13651874	3471
chr3L_Mrandom_184	5.80095056	47	14.1484098	3L	13669583	5362
chr3L_Mrandom_193	3.783261205	39	11.74016983	3L	12787062	1733
chr3L_Mrandom_197	9.132549175	69	20.7710697	3L	13713254	6788

chr3L_Mrandom_210	2.75112019	39	11.74016983	3L	15971934	4685
chr3L_Mrandom_217	2.447474425	21	6.321629909	3L	18832780	1305
chr3L_Mrandom_225	2.308751134	44	13.24531981	X	9678530 2869	
chr3L_Mrandom_259	4.365589581	49	14.75046979	3R	153471 7504	
chr3L_Mrandom_275	8.552386999	61	18.36282974	3R	1402855 21355	
chr3L_Mrandom_279	3.958174573	36	10.83707984	3R	741744 1379	
chr3L_Mrandom_282	3.069984731	39	11.74016983	3L	18834769	1383
chr3L_Mrandom_283	15.46052202	75	22.57724967	3R	253789 16854	
chr3R_Mrandom_001	2.878101981	30	9.03089987	3R	4081586 9263	
chr3R_Mrandom_002	8.486549932	86	25.88857963	3R	21776158	80867
chr3R_Mrandom_003	2.042816164	21	6.321629909	3R	555409 1793	
chr3R_Mrandom_004	7.744582974	87	26.18960962	3R	153471 75460	
chr3R_Mrandom_011	6.92657441	44	13.24531981	3R	153471 4595	
chr3R_Mrandom_014	2.792130696	19	5.719569918	3R	2747847 1280	
chr3R_Mrandom_017	2.201735354	29	8.729869874	3R	3683940 1205	
chr3R_Mrandom_057	7.33200992	64	19.26591972	3R	15733251	10448
chr3R_Mrandom_058	7.44728812	69	20.7710697	3R	15748017	7760
chr3R_Mrandom_086	3.60450943	38	11.43913984	3R	14771103	5697
chr3R_Mrandom_090	8.767634472	60	18.06179974	3R	14271928	16068
chr3R_Mrandom_093	2.979395788	31	9.331929866	3R	14268588	7632
chr3R_Mrandom_104	3.873265078	26	7.826779887	3R	15434211	1030
chr3R_Mrandom_120	2.105560755	16	4.816479931	3R	10291175	1160
chr3R_Mrandom_123	2.394676299	33	9.933989857	3R	11700645	1055
chr3R_Mrandom_129	2.240058114	8	2.408239965	3R	10785063	1672
chr3R_Mrandom_144	2.984885425	31	9.331929866	3R	10477021	1598
chr3R_Mrandom_150	4.780059835	41	12.34222982	3R	10669370	7818
chr3R_Mrandom_154	11.51218879	72	21.67415969	3R	10477531	8851
chr3R_Mrandom_157	2.407718083	11	3.311329952	3R	5693504 2118	
chr3R_Mrandom_186	8.242273233	62	18.66385973	3R	9879876 7503	
chr3R_Mrandom_242	11.89072701	75	22.57724967	3R	17724695	8782
chr3R_Mrandom_256	3.278311384	22	6.622659905	3R	20924672	4152
chr3R_Mrandom_275	2.576413744	21	6.321629909	3R	15339131	2695
chr3R_Mrandom_276	3.412318875	22	6.622659905	3R	20707552	1300
chr3R_Mrandom_289	3.758169976	38	11.43913984	3R	20464224	4125
chr3R_Mrandom_322	2.028340457	33	9.933989857	3R	23529249	5261
chr3R_Mrandom_340	4.745862421	56	16.85767976	3R	23120428	7011
chr3R_Mrandom_362	2.284145723	18	5.418539922	3R	26400470	1590
chr4_Mrandom_001	11.37839367	76	22.87827967	4	584271 9594	
chr4_Mrandom_002	12.83044553	85	25.58754963	4	263332 11590	
chr4_Mrandom_004	8.870235413	66	19.86797971	4	601940 6143	
chr4_Mrandom_007	7.586682557	76	22.87827967	4	853765 6500	
chr4_Mrandom_012	2.847804868	27	8.127809883	4	688583 6356	
chr4_Mrandom_015	2.226844859	17	5.117509926	4	892896 4531	
chr4_Mrandom_018	7.305756676	60	18.06179974	4	401309 5467	
chr4_Mrandom_019	15.54947595	76	22.87827967	4	818865 19470	
chr4_Mrandom_020	2.407129854	8	2.408239965	X	10002229	6209
chrX_Mrandom_001	16.43102184	81	24.38342965	X	12193639	47258
chrX_Mrandom_014	20.7434582	86	25.88857963	X	14557708	101595
chrX_Mrandom_015	7.936194073	56	16.85767976	X	12193639	15993
chrX_Mrandom_019	3.994311218	27	8.127809883	X	15396013	11609
chrX_Mrandom_021	16.51997929	88	26.49063962	X	15653877	64735
chrX_Mrandom_022	24.27736236	93	27.9957896	X	7671683 178279	
chrX_Mrandom_023	3.469754657	27	8.127809883	X	4242668 10648	
chrX_Mrandom_034	2.164755172	29	8.729869874	X	4473613 13916	
chrX_Mrandom_037	13.42046705	71	21.37312969	X	486013 12248	
chrX_Mrandom_041	11.74632578	72	21.67415969	X	143522 7168	
chrX_Mrandom_052	3.42651383	23	6.9236899	X	113337 7901	
chrX_Mrandom_069	2.00114947	17	5.117509926	X	489313 5970	
chrX_Mrandom_074	5.786865851	47	14.1484098	X	446454 5594	
chrX_Mrandom_082	2.250342324	26	7.826779887	X	618042 3577	
chrX_Mrandom_1010	7.166751288	43	12.94428981	X	15364330	6780
chrX_Mrandom_1011	2.470204235	25	7.525749892	X	15741485	1693
chrX_Mrandom_1013	10.0243661	55	16.55664976	X	15731348	9961
chrX_Mrandom_1021	3.06054731	36	10.83707984	X	15343217	16095
chrX_Mrandom_1024	7.717467497	61	18.36282974	X	15940505	10654
chrX_Mrandom_1028	4.310001308	43	12.94428981	X	15876486	18975
chrX_Mrandom_1035	3.022925291	36	10.83707984	X	15653877	7087
chrX_Mrandom_1045	4.199669099	40	12.04119983	X	15623518	1346
chrX_Mrandom_1052	2.103088653	17	5.117509926	3R	13383481	14449

chrX_Mrandom_1056	2.126008462	46	13.8473798	2L	21298617	32283
chrX_Mrandom_1061	4.750481352	59	17.76076974	X	15731348	13722
chrX_Mrandom_1062	3.900357788	38	11.43913984	X	15370980	7088
chrX_Mrandom_112	2.467712387	16	4.816479931	X	626455	12236
chrX_Mrandom_128	5.428062885	37	11.13810984	X	1448020	13226
chrX_Mrandom_140	5.898839082	32	9.632959861	X	1652919	11827
chrX_Mrandom_154	2.592151333	31	9.331929866	X	1448020	6974
chrX_Mrandom_163	5.113841145	48	14.44943979	X	1689862	7513
chrX_Mrandom_165	2.46227208	35	10.53604985	X	1722258	7184
chrX_Mrandom_204	3.925765676	27	8.127809883	X	3467655	2675
chrX_Mrandom_216	3.261643284	34	10.23501985	X	1956992	10339
chrX_Mrandom_217	10.59333671	65	19.56694972	X	2861561	17795
chrX_Mrandom_245	2.317141479	27	8.127809883	X	3556072	1956
chrX_Mrandom_255	3.621834658	28	8.428839879	X	3944265	5453
chrX_Mrandom_256	2.475234223	17	5.117509926	X	3556041	4432
chrX_Mrandom_259	5.455158012	28	8.428839879	X	3638749	4069
chrX_Mrandom_268	3.129513547	26	7.826779887	X	4140296	8401
chrX_Mrandom_276	3.681216075	32	9.632959861	X	4016891	10762
chrX_Mrandom_277	7.184371074	46	13.8473798	X	4016891	11595
chrX_Mrandom_278	2.325652062	19	5.719569918	X	4016891	1628
chrX_Mrandom_282	6.939135808	50	15.05149978	X	4201040	6347
chrX_Mrandom_284	3.085564358	23	6.9236899	X	7252516	12645
chrX_Mrandom_288	2.401280139	36	10.83707984	X	4010791	4077
chrX_Mrandom_334	3.394543996	36	10.83707984	X	6401342	7472
chrX_Mrandom_335	2.271975122	28	8.428839879	X	6400653	4596
chrX_Mrandom_341	3.411503073	37	11.13810984	X	6416733	1889
chrX_Mrandom_358	2.411148848	20	6.020599913	2R	12671668	5578
chrX_Mrandom_366	3.060910445	22	6.622659905	X	7359036	6380
chrX_Mrandom_368	5.164648784	43	12.94428981	X	5319054	9272
chrX_Mrandom_372	2.372675679	11	3.311329952	X	7263009	3791
chrX_Mrandom_391	8.885682671	64	19.26591972	X	6400748	9550
chrX_Mrandom_397	3.575746084	29	8.729869874	X	7385600	4118
chrX_Mrandom_398	3.530108648	31	9.331929866	X	6416733	1760
chrX_Mrandom_402	2.55984847	33	9.933989857	X	1918208	5460
chrX_Mrandom_410	2.817551241	22	6.622659905	X	2665435	7943
chrX_Mrandom_421	3.574119225	24	7.224719896	X	7385600	7376
chrX_Mrandom_423	2.444096877	22	6.622659905	X	7385600	14418
chrX_Mrandom_434	2.185341317	11	3.311329952	X	5940026	4089
chrX_Mrandom_436	5.307294743	34	10.23501985	X	6400653	8858
chrX_Mrandom_440	7.491267891	50	15.05149978	X	6822267	8525
chrX_Mrandom_444	2.090645408	32	9.632959861	X	7359036	4617
chrX_Mrandom_472	6.334555464	34	10.23501985	X	7385600	27792
chrX_Mrandom_484	6.389991927	56	16.85767976	X	7658807	10053
chrX_Mrandom_489	2.487328392	27	8.127809883	X	7385600	3665
chrX_Mrandom_502	2.470878825	15	4.515449935	X	7385600	11234
chrX_Mrandom_512	3.272113355	21	6.321629909	X	7671683	1583
chrX_Mrandom_513	2.788594958	37	11.13810984	X	6743357	3318
chrX_Mrandom_523	13.74410833	70	21.0720997	X	7663603	11564
chrX_Mrandom_525	2.612015654	22	6.622659905	X	9343620	4076
chrX_Mrandom_531	2.918318716	31	9.331929866	X	4758388	10342
chrX_Mrandom_537	3.932990041	27	8.127809883	X	7385600	4260
chrX_Mrandom_541	5.021240822	27	8.127809883	X	9649067	10727
chrX_Mrandom_572	3.206716934	20	6.020599913	X	8160681	8285
chrX_Mrandom_584	10.71785706	73	21.97518968	X	8160681	16529
chrX_Mrandom_586	3.115590562	26	7.826779887	X	9682331	4363
chrX_Mrandom_589	11.59914802	58	17.45973975	X	8160770	7254
chrX_Mrandom_590	5.741795875	44	13.24531981	X	8208736	8096
chrX_Mrandom_591	5.113713374	44	13.24531981	X	8160992	8609
chrX_Mrandom_606	3.601923762	33	9.933989857	X	9377600	3210
chrX_Mrandom_607	3.478662595	37	11.13810984	X	9508665	5892
chrX_Mrandom_612	3.36285707	18	5.418539922	X	8208736	4008
chrX_Mrandom_618	6.179309228	43	12.94428981	X	9276078	36515
chrX_Mrandom_631	7.176585749	46	13.8473798	X	9570389	5858
chrX_Mrandom_632	2.565982426	24	7.224719896	X	9570389	5092
chrX_Mrandom_647	5.439408145	40	12.04119983	X	9635988	8015
chrX_Mrandom_661	2.033149003	11	3.311329952	X	9742926	6426
chrX_Mrandom_672	11.58327989	65	19.56694972	X	10281212	20258
chrX_Mrandom_675	2.491706572	9	2.709269961	X	8208696	3528
chrX_Mrandom_686	4.063689579	26	7.826779887	X	12145900	14739

chrX_Mrandom_724	2.098678679	20	6.020599913	X	12176730	2216
chrX_Mrandom_770	8.201607472	47	14.1484098	X	12139393	9886
chrX_Mrandom_775	2.04281298	21	6.321629909	X	12145900	4776
chrX_Mrandom_777	6.032161052	37	11.13810984	X	12145900	9749
chrX_Mrandom_778	6.828851107	47	14.1484098	X	11991380	8194
chrX_Mrandom_779	2.542255488	27	8.127809883	X	12176107	10644
chrX_Mrandom_785	7.937289863	88	26.49063962	X	12195621	48010
chrX_Mrandom_786	2.91424579	20	6.020599913	X	13583362	6586
chrX_Mrandom_787	2.259962635	16	4.816479931	X	13578240	7227
chrX_Mrandom_793	10.79314016	69	20.7710697	X	12573212	9952
chrX_Mrandom_794	10.54897131	60	18.06179974	X	12396276	28794
chrX_Mrandom_803	3.963876803	34	10.23501985	X	12572649	11635
chrX_Mrandom_821	3.554372743	21	6.321629909	X	12320562	4811
chrX_Mrandom_823	2.041449823	7	2.10720997	2L	5052716	1275
chrX_Mrandom_848	4.526509895	43	12.94428981	X	13586035	13570
chrX_Mrandom_854	5.14490094	40	12.04119983	X	14557708	9917
chrX_Mrandom_858	6.250095902	41	12.34222982	X	14850201	6210
chrX_Mrandom_868	5.677763723	55	16.55664976	X	13936530	5113
chrX_Mrandom_893	2.826346755	30	9.03089987	X	14888069	7708
chrX_Mrandom_926	4.220559283	41	12.34222982	X	14972654	3757
chrX_Mrandom_928	7.514031763	56	16.85767976	X	14790349	9918
chrX_Mrandom_945	3.068487799	18	5.418539922	X	13343089	2424
chrX_Mrandom_955	5.206875341	47	14.1484098	X	14972105	5896
chrX_Mrandom_973	2.101027768	14	4.214419939	3L	10841591	4921
chrX_Mrandom_987	5.163612803	26	7.826779887	X	15364151	10990
chrX_Mrandom_993	3.904909117	28	8.428839879	X	16098585	5115
chrX_Mrandom_998	3.766741627	27	8.127809883	X	15867265	10755
chrXh_Mrandom_002	2.950821012	41	12.34222982	X	16098585	10104
chrXh_Mrandom_005	10.68981528	83	24.98548964	X	15653877	57159
chrU_M_1003	4.27806832	16	4.816479931	2L	10505181	4817
chrU_M_1011	4.9406394	42	12.64325982	4	475364	2823
chrU_M_1048	4.079406612	46	13.8473798	4	445123	4710
chrU_M_1158	2.187762516	52	15.65355977	2L	10283145	3923
chrU_M_1163	11.12377341	86	25.88857963	3L	21384738	59025
chrU_M_1194	10.43608352	88	26.49063962	2L	19595887	40927
chrU_M_1255	11.33380588	89	26.79166961	3L	21077626	63310
chrU_M_1316	2.850753983	90	27.09269961	3R	6565377	56439
chrU_M_1341	16.21960422	89	26.79166961	3L	19333977	35657
chrU_M_1374	3.514335895	45	13.5463498	3L	9464814	1818
chrU_M_1377	4.235412247	25	7.525749892	X	15013890	3436
chrU_M_1399	4.712176737	42	12.64325982	3R	1402855	37926
chrU_M_1464	2.755064523	26	7.826779887	X	7385600	3728
chrU_M_1482	4.553105763	42	12.64325982	4	595099	3288
chrU_M_1513	3.083485889	37	11.13810984	4	458592	2906
chrU_M_1545	2.180794303	30	9.03089987	4	678259	2779
chrU_M_1548	2.13980229	22	6.622659905	4	687919	1682
chrU_M_1582	2.073796855	8	2.408239965	X	15442568	2381
chrU_M_1644	17.16282992	87	26.18960962	3L	20601042	32717
chrU_M_165	2.005695128	46	13.8473798	2L	8261791	7144
chrU_M_1656	2.213117489	58	17.45973975	2L	10145788	31364
chrU_M_1729	3.547848394	83	24.98548964	3R	153471	27910
chrU_M_1789	2.007776408	32	9.632959861	4	165837	3777
chrU_M_1835	4.254796241	58	17.45973975	2L	19642849	29498
chrU_M_1928	18.69062595	83	24.98548964	2R	960254	25780
chrU_M_1962	2.867268399	73	21.97518968	3R	153471	30439
chrU_M_2008	8.469374718	73	21.97518968	2R	683677	25494
chrU_M_2079	13.05490538	75	22.57724967	3L	22438320	34821
chrU_M_2099	2.764640807	74	22.27621968	2L	10283145	24768
chrU_M_2110	12.03544685	82	24.68445964	2R	682986	30368
chrU_M_2151	5.997591878	77	23.17930967	2L	20195522	28356
chrU_M_2229	2.507279619	71	21.37312969	2L	10328358	11016
chrU_M_2288	2.761944597	24	7.224719896	2L	11766009	11690
chrU_M_2295	3.701217172	63	18.96488973	2L	10234933	15552
chrU_M_2316	6.522075406	68	20.47003971	3L	18130440	16958
chrU_M_2372	3.493178064	20	6.020599913	2R	7945813	18205
chrU_M_2407	2.851417565	13	3.913389944	2R	19251935	890
chrU_M_2422	2.675910056	43	12.94428981	X	16098585	14337
chrU_M_2472	2.041792088	73	21.97518968	2L	11589352	1752
chrU_M_2476	4.151201612	40	12.04119983	X	16098585	15422

chrU_M_2514	2.150951212	9	2.709269961	3R	1276957	1259	
chrU_M_2561	3.3691503	30	9.03089987	3L	18221577		1779
chrU_M_2568	2.156368519	10	3.010299957	3L	1985650	1284	
chrU_M_2593	2.179442034	51	15.35252978	2L	21104173		18352
chrU_M_2644	12.53395686	68	20.47003971	2L	21440028		11786
chrU_M_2700	2.743859916	81	24.38342965	2L	21028003		19149
chrU_M_2808	3.295422555	72	21.67415969	2L	10241780		15371
chrU_M_2901	17.86710147	86	25.88857963	2L	21677421		14992
chrU_M_2909	2.837688612	30	9.03089987	2L	10262473		17365
chrU_M_2915	2.246793749	69	20.7710697	2L	10195833		11767
chrU_M_2924	2.633042532	34	10.23501985	2R	1622026	8649	
chrU_M_2931	2.119153661	23	6.9236899	2L	19492193		1045
chrU_M_2954	14.12099577	89	26.79166961	3L	19424487		14816
chrU_M_2991	2.202289537	23	6.9236899	3L	22438320		12057
chrU_M_3080	2.218088755	23	6.9236899	3R	20159586		1060
chrU_M_3086	2.708020971	9	2.709269961	X	15305252		1221
chrU_M_3158	2.81039041	41	12.34222982	2L	8171204	8858	
chrU_M_3297	2.16213576	23	6.9236899	3R	19197383		1802
chrU_M_3488	5.308790721	60	18.06179974	2R	190702	9806	
chrU_M_3687	3.66890936	80	24.08239965	2L	21829378		16075
chrU_M_3725	3.643672372	44	13.24531981	3L	16003926		9864
chrU_M_3743	5.307260135	47	14.1484098	3R	1268118	9974	
chrU_M_3744	3.668909166	40	12.04119983	2R	16373611		1260
chrU_M_3775	2.099427894	32	9.632959861	2L	13650696		12808
chrU_M_3809	2.028063229	52	15.65355977	2L	10346774		9225
chrU_M_3839	5.175092113	63	18.96488973	2R	133252	8942	
chrU_M_3992	3.172340888	54	16.25561977	X	16098585		1034
chrU_M_4016	3.300079902	41	12.34222982	3R	741744	13169	
chrU_M_4235	2.411807407	21	6.321629909	2L	10288016		10397
chrU_M_4498	2.520838111	33	9.933989857	3L	19377562		10148
chrU_M_4508	3.173294069	38	11.43913984	2R	1230003	9580	
chrU_M_4546	2.926469653	14	4.214419939	2L	20122367		7783
chrU_M_4691	2.185354249	55	16.55664976	3R	20041814		9721
chrU_M_4776	2.341109849	8	2.408239965	3L	19652083		9441
chrU_M_4848	2.406906949	17	5.117509926	3L	9248318	866	
chrU_M_4861	7.714706607	61	18.36282974	3L	19340034		9374
chrU_M_5047	2.810775745	23	6.9236899	4	652651	865	
chrU_M_5070	4.174714749	70	21.0720997	2L	13459792		7110
chrU_M_534	2.011192417	27	8.127809883	4	570933	1171	
chrU_M_5463	4.036105981	39	11.74016983	2R	4177139	1034	
chrU_M_5524	2.399065053	22	6.622659905	2L	9089924	1327	
chrU_M_5684	4.417146324	47	14.1484098	3L	20079094		6865
chrU_M_5690	4.269534516	36	10.83707984	2L	12552508		942
chrU_M_5804	3.210401866	23	6.9236899	2L	20859218		962
chrU_M_5848	3.386428691	30	9.03089987	4	460778	1586	
chrU_M_5866	3.418584399	32	9.632959861	2L	21104173		6590
chrU_M_6098	2.171860518	17	5.117509926	X	14850201		885
chrU_M_6201	3.577008205	42	12.64325982	3R	1402855	7373	
chrU_M_6225	3.909779611	38	11.43913984	2R	190702	9242	
chrU_M_6502	3.089713438	22	6.622659905	2L	9275280	734	
chrU_M_6608	2.088161547	23	6.9236899	2L	11713248		7900
chrU_M_668	2.687113534	16	4.816479931	3L	22438320		6870
chrU_M_6729	2.53565301	37	11.13810984	2L	11589352		11596
chrU_M_677	2.474682152	25	7.525749892	3R	17512583		4850
chrU_M_6813	2.933957473	21	6.321629909	X	10640525		688
chrU_M_6873	2.247604609	12	3.612359948	2R	9958682	866	
chrU_M_705	6.596263304	60	18.06179974	2R	1976565	7426	
chrU_M_706	2.488002953	18	5.418539922	3L	19388401		4889
chrU_M_707	3.553106177	33	9.933989857	2R	1431074	6466	
chrU_M_7133	3.245232722	77	23.17930967	2L	10283145		6767
chrU_M_715	2.270500082	40	12.04119983	3R	19009381		6114
chrU_M_725	2.023245887	45	13.5463498	2L	13216914		5497
chrU_M_742	2.251528637	15	4.515449935	X	15680348		5706
chrU_M_792	4.322677986	60	18.06179974	3R	220594	6447	
chrU_M_869	7.251434239	65	19.56694972	4	688694	6609	
chrU_M_927	16.49603285	88	26.49063962	3R	153471	81372	
chrU_M_967	3.225985719	36	10.83707984	2R	1890345	4787	
chrU_M_976	4.425040225	90	27.09269961	3R	153471	74428	

S4. Human-chimpanzee F1-backcross simulation.

To generate human-chimpanzee F1-backcross individuals, we first created human and chimpanzee versions of chromosome 1 by stitching together syntenic pairwise alignments from <http://hgdownload.cse.ucsc.edu/goldenPath/hg19/vsPanTro2/syntenicNet/chr1.hg19.panTro2.synNet.axt.gz> and padding the junctions with 101 Ns (the maximum read length in our *Drosophila* dataset). To create 96 recombinant individuals, we randomly selected breakpoints on chromosome 1 and created chimeric chromosomes such that one side flanking this breakpoint was composed of human sequence and the other side, chimpanzee sequence. To this, we added a second copy of chromosome 1 entirely composed of human sequence and rendering the chimp-derived part of chromosome 1 heterozygous. The remainder of the genome (chromosomes 2-23) is assumed to be diploid human sequence and we used a standard human genome reference sequence (<http://hgdownload.cse.ucsc.edu/goldenPath/hg19/bigZips/chromFa.tar.gz>). Thus, each recombinant individual is heterozygous (human-chimp) for part of chromosome 1 and homozygous for the rest of the genome.

We next simulated short-read sequences from these 96 “recombinant” diploid genomes using a custom Python script (available on request). This script generates single read data from MseI sites, mimicking reads from a MSG library, and the number of reads for each individual was assigned by sampling from the distribution of reads observed in our *Drosophila* experiment (without replacement). Simulated reads were assumed to be free of DNA sequencing error, though we varied the sequence length randomly from 75-101 bp to mimic quality trimming of real data.

We analysed reads from these 96 recombinant genomes using the same MSG pipeline used on our *Drosophila* data. We assumed no uncertainty in the parental

genomes (i.e. we set $\gamma = 0$) and we set the expected transition probability (r) to one recombination event per chromosome. All reads were mapped to standard human and chimp reference genomes (human: <http://hgdownload.cse.ucsc.edu/goldenPath/hg19/bigZips/chromFa.tar.gz>; chimpanzee: <http://hgdownload.cse.ucsc.edu/goldenPath/panTro2/bigZips/chromFa.tar.gz>). Using the stringent criterion we used to define breakpoints in our *Drosophila* data (i.e. a change in ancestry from 95% to 5%, we detected 95 of 96 simulated breakpoints on chromosome 1 and we confirmed that all true breakpoints we detected occurred within inferred breakpoint intervals. We failed to detect one recombination event at position 248776535, which is <500 kb from the telomere of arm 1q. However, on closer inspection, a change in ancestry probability does occur at this breakpoint from <5%, 48 kb to the left of the true breakpoint, to 93%, 57 kb to the right of it. No breakpoints were found on other chromosomes.

Figure S4.1 shows the analysis of an individual with the median number (A) and the lowest number (B) of reads. Figure S4.2 shows the distribution of breakpoint widths and we found the median breakpoint width to be 196 kb. This is significantly wider than in our *Drosophila* data (the most appropriate comparison is Figure 3C 1% divergence where the median breakpoint width is 77 kb), which is expected given that the same number of reads are being applied to a ~20-fold larger genome. In fact, we found a strong log-linear relationship between the number of reads and breakpoint resolution (Pearson's $R^2 = -0.84$, Figure S4.3). We conclude that genome size (or "complexity") *per se* is unlikely to limit the applicability of our method.

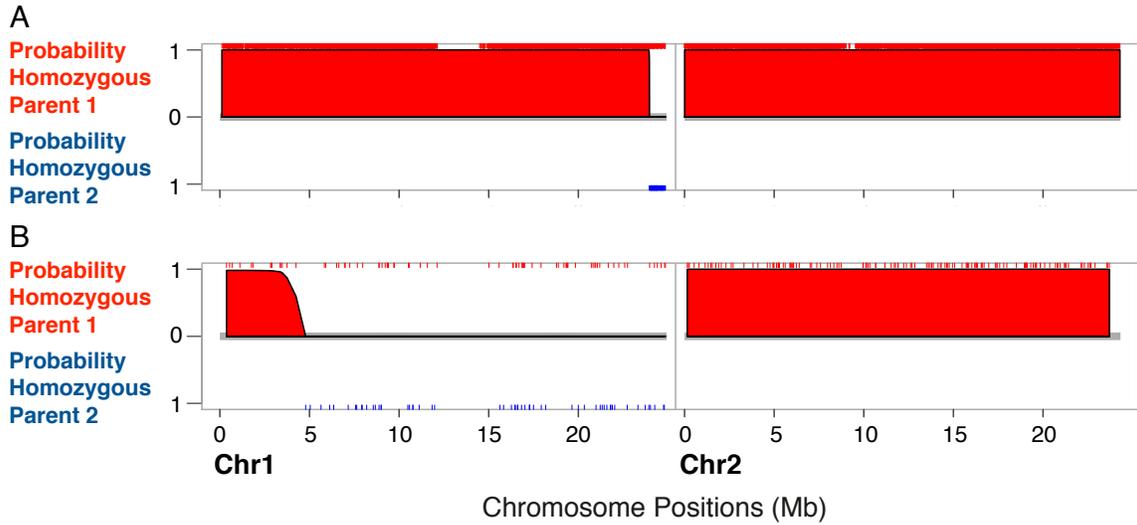


Figure S4.1. Ancestry plots of human chromosome 1 and 2 for (A) the median-covered individual (F10_CTTATG) and (B) the individual with the least number of reads (C12_CACCGG).

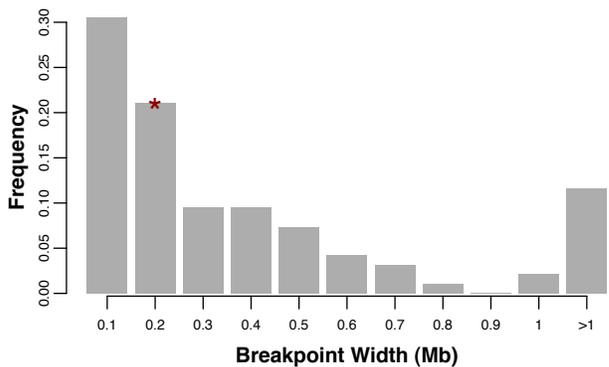


Figure S4.2 Distribution of breakpoint widths (Mb) for 95 of the 96 breakpoints detected. Breakpoint widths are defined as the interval over which the probability of a given ancestry changes from 0.95 to 0.05. Most of the breakpoints resolved to >1Mb span the centromere, which has no informative markers (roughly positions 12-15 Mb, see Figure S4.1A). The median breakpoint width is indicated by the red asterisk.

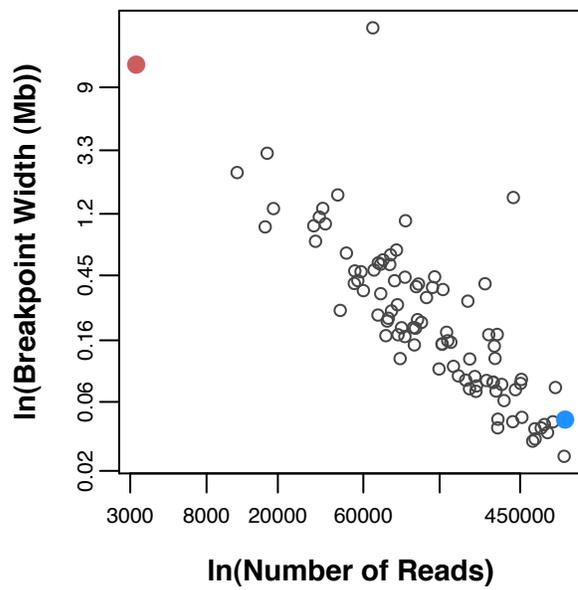


Figure S4.3. The relationship between the number of reads and inferred breakpoint widths for 95 of 96 simulated recombination events on chromosome 1 (Pearson's $R^2 = -0.84$). Individuals with the fewest and most reads are indicated with red and blue circles, respectively.

S5. Drosophila backcross experiment

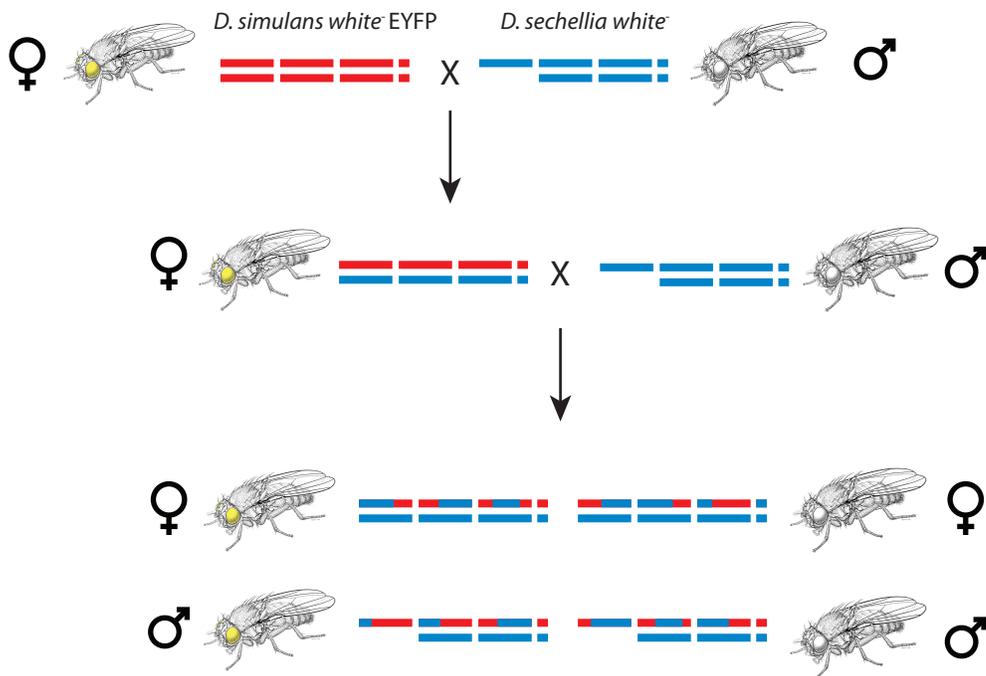


Figure S5. Crossing scheme used to generate backcross individuals used to map the EYFP marker. A strain of *D. simulans* was generated that carried a EYFP marker on the X chromosome. This homozygous strain was crossed to *D. sechellia*. The F1 offspring that resulted from this cross were backcrossed to *D. sechellia* males. The backcross progeny from this cross were scored for sex and for the presence or absence of EYFP expression in the eyes.

S6. The 96 barcodes used in this study.

AAATAG	TATATG	TATGCG	AACGAG	TAGCTG	ATATGG	G TTCAG	ATCTTG
TAATTG	CATACG	TACAAG	TACGTG	CAGCCG	CTACAG	ATTCTG	TTCTCG
CAATCG	GATAGG	CACATG	CACGCG	GAGCGG	G TACTG	TTTCCG	CTCTGG
GAATGG	TATTAG	GACACG	CAGAAG	TAGGAG	ATACCG	CTTCGG	ATCCAG
TAACAG	GATTCTG	AACAGG	GAGATG	CAGGTG	TTACGG	ATTGAG	TTCTG
CAACTG	AATTGG	CACTAG	AAGACG	GAGGCG	G TAGAG	TTTGTG	GTCCGG
GAACCG	CATCAG	GACTTG	TAGAGG	TTAATG	ATAGTG	CTTGCG	TTGAG
AAACGG	GATCTG	AACTCG	GAGTAG	CTAACG	TTAGCG	CTCAAG	CTCGTG
CAAGAG	AATCCG	TACTGG	AAGTTG	GTAAGG	TTTAAG	GTCATG	GTGCGG
GAAGTG	TATCGG	GACCAG	TAGTCG	TTATAG	CTTATG	ATCACG	GTGAAG
AAAGCG	GATGAG	AACCTG	CAGTGG	CTATTG	GTTACG	TTCAGG	ATGATG
AATAAG	AATGTG	CACCGG	AAGCAG	GTATCG	ATTAGG	GTCTAG	TTGACG

S7. Representation of barcodes among individuals

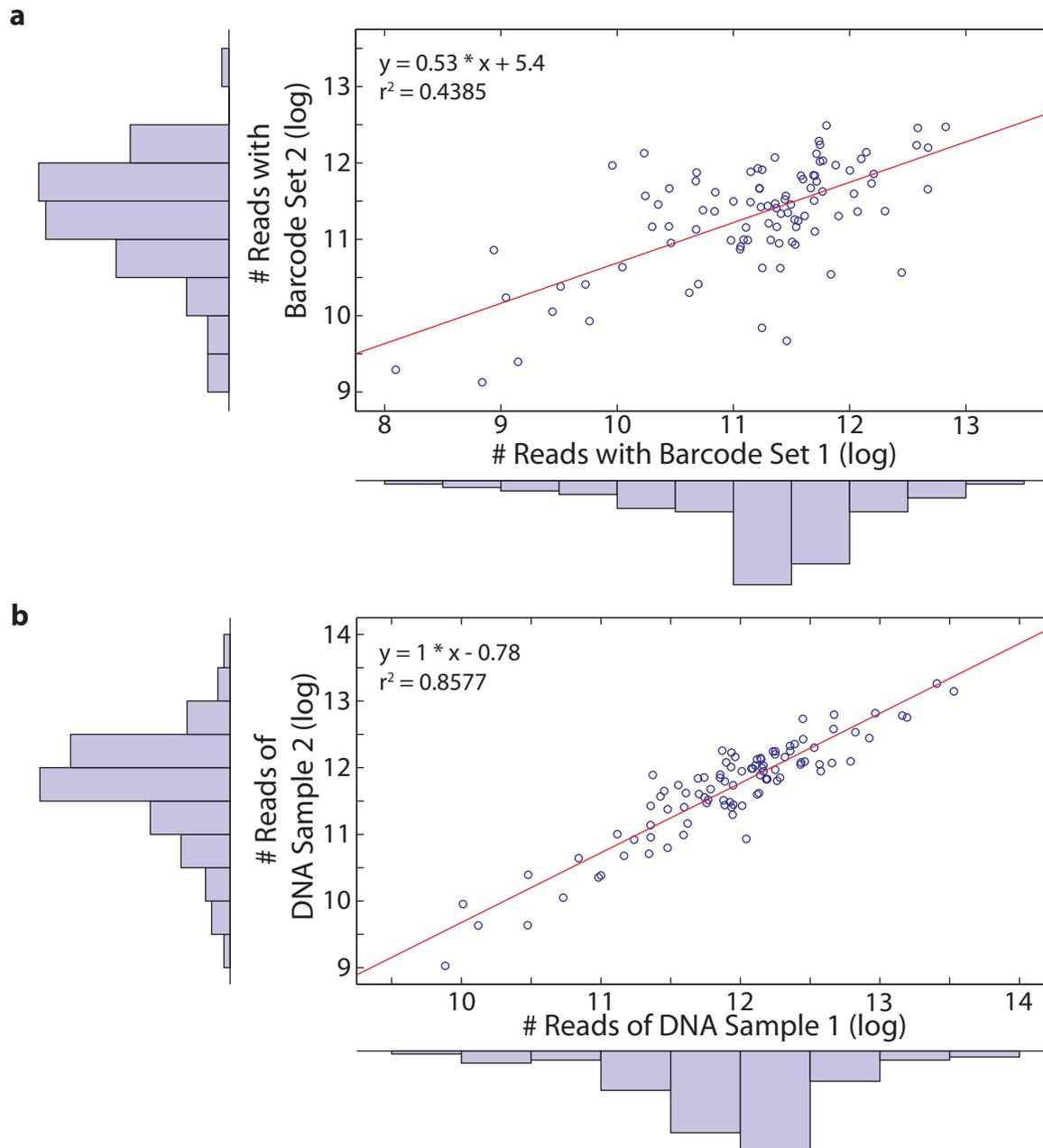


Figure S7. Both barcode identity and DNA quality influence read representation in MSG libraries. **(a)** Plot of the barcode representation for two MSG libraries made with different sets of 96 barcodes for a single set of 96 genomic DNA (gDNA) samples. The correlation indicates that one or more characteristics of the gDNA samples, what we call “DNA quality”, influences the final number of sequenced reads per barcode. **(b)** Barcode representation for two MSG libraries made with the same 96 barcodes and two different gDNA samples. For each library, each barcode linker was ligated onto a sample containing an equimolar, equal volume aliquot of the same gDNA sample. The correlation between libraries indicates that linker identity strongly influences the final number of sequenced reads per barcode. Note that the plot in **a** does not control for variance due to barcode identity, so the true effect of DNA quality is probably stronger than shown by the correlation in **a**.

S8. Hidden Markov Model (HMM) to estimate ancestry.

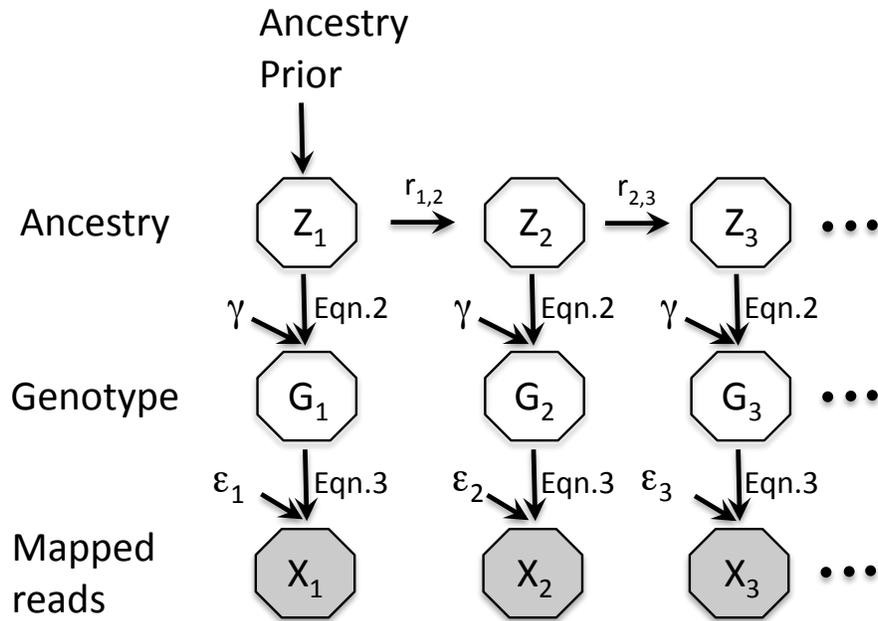


Figure S8. Graphical model depiction of the HMM used to infer ancestry. The figure depicts the structure of the HMM for the first three markers along a chromosome. Unshaded nodes represent unobserved quantities, whereas shaded nodes represent observed data (the base calls for mapped sequence reads overlapping a given marker). The emission probabilities for a given marker depend on an unknown quantity of interest (ancestry at that marker) via a further unknown quantity (the genotype, which is not directly of interest), giving rise to the summation in Equation 1. $r_{m,m+1}$ indicates the ancestry state transition probabilities from marker m to $m+1$. See Methods for a description of other equations and symbols.

S9. Identifying misassembled regions of the *D. simulans* genome

Our approach assumes that one of the reference genomes is correctly assembled. To evaluate this assumption, we generated a matrix of pairwise LOD estimates among loci (Methods and Figure S9.1). Based on this analysis, we conclude that most of the *D. simulans* reference genome conforms to the predicted linkage relationships. However, we identified six genomic regions that produced ancestry calls that were inconsistent with flanking regions (Figure S9.2). Such regions are apparent as discontinuities in the sequence of LOD scores between adjacent markers (Figure S9.1). Table S9.1 lists all of the regions that we detected. One example is a ~1 Mb region on chromosome 2R. Our linkage tests determined that this region is in fact located near the telomere of chromosome 3L (Figure S9.2). Indeed, examination of the genes encoded in this region reveals a block of >350 kb encoding genes orthologous to genes on *D. melanogaster* chromosome 3L. All six regions (Table S9.1) were masked in subsequent analyses. Examination of the annotation (.gff) files associated with the genome assembly (retrieved from Flybase) revealed a number of other regions that have been incorrectly assembled to chromosome arms (Table S9.2), but we failed to detect most of these apparent errors through linkage analysis, probably because they are located in misassembled regions that are too small to be distinguished from genotyping error.

Table S9.2

A list of regions that map discordantly between the *D. simulans* assembly and the *D. melanogaster* genome reference sequence
 # derived from the gff for the *D. simulans* genome reference release 1.3 (ftp://ftp.flybase.net/genomes/Drosophila_simulans/dsim_r1.3_FB2010_07/gff)

chr_Dsim	contig_Dsim	start_Dsim	stop_Dsim	chr_Dmel	start_Dmel	stop_Dmel	Notes
2L	10266499	10268452	X	14805876	14807830		
2L	10270396	10275311	X	14810804	14812777		
2L	10999214	10999699	3L	18245188	18245946		
2L	20232720	20234353	2R	10657104	10658417		
2L	20299846	20300157	X	10326498	10326845		
2L	21630954	21632088	2R	2603847	2605312		
2R	3882202	3883050	3L	13081679	13083069		
2R	4621875	4622291	3R	9417998	9418336		
2R	6779351	6781192	3R	8033879	8036079		
2R	8471690	8473688	3L	247495	250314		*confirmed see Table S9.1
2R	8474216	8481704	3L	250229	257950		*confirmed see Table S9.1
2R	8482487	8483090	3L	258350	259097		*confirmed see Table S9.1
2R	8483614	8488356	3L	259286	264378		*confirmed see Table S9.1
2R	8488975	8489561	3L	264782	265599		*confirmed see Table S9.1
2R	8492270	8493159	3L	267764	270340		*confirmed see Table S9.1
2R	8496241	8497365	3L	272210	273370		*confirmed see Table S9.1
2R	8497561	8498894	3L	271640	275021		*confirmed see Table S9.1
2R	8499357	8500565	3L	275164	276661		*confirmed see Table S9.1
2R	8516564	8516848	3L	290727	305292		*confirmed see Table S9.1
2R	8521573	8527566	3L	290727	305292		*confirmed see Table S9.1
2R	8530054	8539812	3L	306207	319880		*confirmed see Table S9.1
2R	8547640	8550369	3L	321232	324717		*confirmed see Table S9.1
2R	8550894	8553274	3L	324985	327987		*confirmed see Table S9.1
2R	8555693	8557587	3L	329822	332306		*confirmed see Table S9.1
2R	8582318	8582686	3L	357981	358486		*confirmed see Table S9.1
2R	8582783	8585414	3L	357851	361383		*confirmed see Table S9.1
2R	8585932	8589521	3L	361478	365681		*confirmed see Table S9.1
2R	8590063	8590554	3L	365664	366437		*confirmed see Table S9.1
2R	8591818	8601097	3L	366524	377316		*confirmed see Table S9.1
2R	8615384	8616176	3L	390941	392824		*confirmed see Table S9.1
2R	8651806	8652279	3L	433230	433891		*confirmed see Table S9.1
2R	8653703	8727410	3L	434258	540589		*confirmed see Table S9.1
2R	8761911	8762932	3L	540851	542147		*confirmed see Table S9.1
2R	8763098	8763730	3L	542122	542916		*confirmed see Table S9.1
2R	8769495	8814396	3L	543070	576065		*confirmed see Table S9.1
3L	4426029	4434446	2L	17501467	17525517		
3L	6733464	6734340	X	17604819	17645728		
3L	9523741	9524778	2R	20136578	20138826		
3L	20381490	20382451	2L	5051193	5053209		
3L	20384075	20386645	2L	5047818	5050877		
3L	20523086	20524147	2L	5718121	5720896		
3R	5290673	5293143	X	20894417	20896915		
3R	5820760	5821392	X	8292973	8297975		
3R	12095492	12096283	4	1144800	1145807		
3R	14631731	14632538	X	19852422	19853421		
X	3126370	3130982	2R	953796	957715		*confirmed see Table S9.1
X	3156780	3159925	2R	978092	982595		*confirmed see Table S9.1
X	3171579	3175408	2R	1004715	1015817		*confirmed see Table S9.1
X	11941312	11941568	3L	19945280	19945890		
X	11943043	11943608	3L	19942282	19944235		
X	16222116	16223702	2R	18691027	18694490		*confirmed see Table S9.1
X	17019891	17022024	3R	5377765	5379469		* possibly confirmed see Table S9.1?

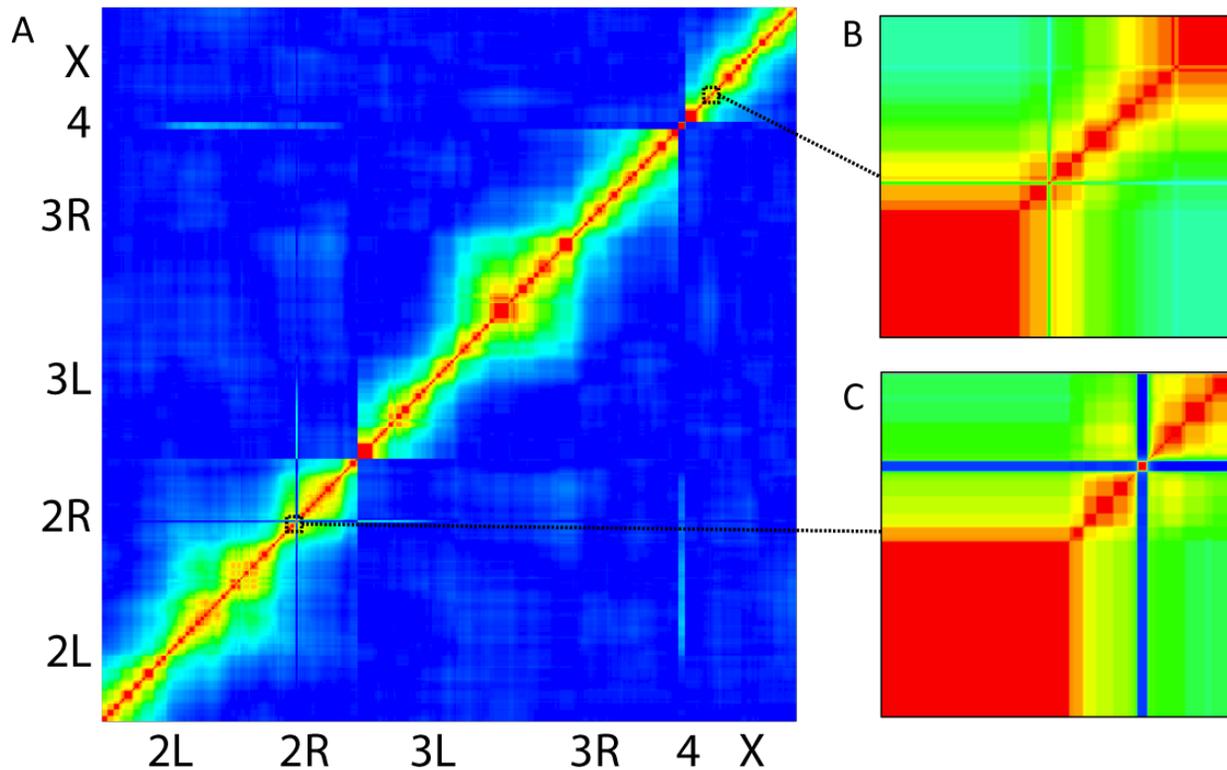


Figure S9.1. A. Genetic correlation between all marker pairs. Each informative marker has been plotted in physical distance and the extent of correlation between pairs of sites (LOD) is plotted in color (blue = low LOD; red = high LOD). Markers have been thinned such that those with similar ancestry patterns have been collapsed into one marker. Panels B and C detail regions on X and 2R, respectively, that show aberrant segregation patterns. This analysis genetically maps the region on 2R to the telomere of chromosome 3L, demonstrating that it is due to a misassembly of the *D. simulans* genome (Figure S9.3).

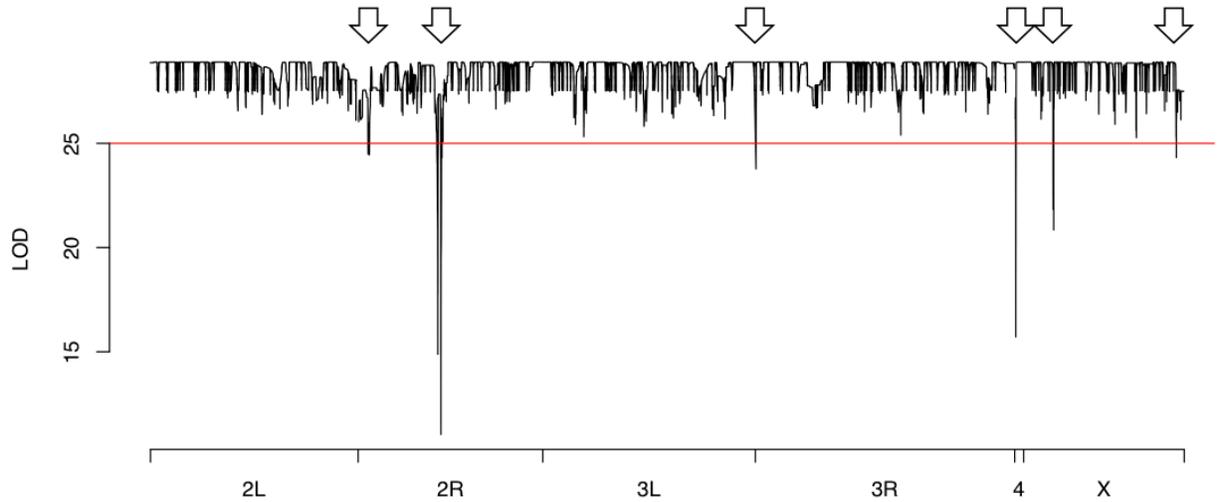


Figure S9.2. Genetic correlation (LOD) between adjacent markers across the *D. simulans* genome. The red line indicates a LOD of 25, which is 3.9 LOD units lower than the maximum possible LOD (28.9) for this experiment. Six regions (indicated with arrows and listed in Table S9) defined by severe discontinuities in LOD between adjacent markers were identified that likely reflect misassembly of the *D. simulans* genome. These regions were masked prior to subsequent analyses.

Table S9.1 Six regions of the *D. simulans* genome that were masked due to anomalous segregation patterns.

Chromosome arm	Start	End	Size
2R	1030000	1230000	200000
2R	8050000	9600000	1550000
3R	0	153395	153395
4	0	163000	163000
X	3100000	3270000	170000
X	16100000	16983271	883271

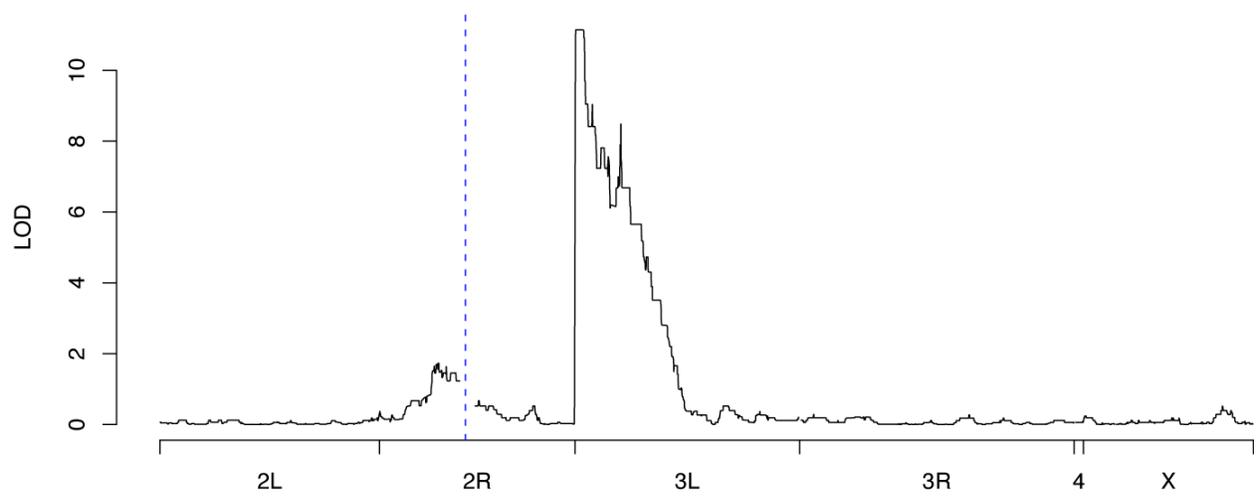


Figure S9.3. Genetic mapping of a ~1.5 Mb region on chromosome 2R to the tip of chromosome 3L. The vertical dotted line represents the approximate midpoint of the region's physical location in the *D. simulans* genome assembly.

Table S9.2

A list of regions that map discordantly between the *D. simulans* assembly and the *D. melanogaster* genome reference sequence

derived from the gff for the *D. simulans* genome reference release 1.3 (ftp://ftp.flybase.net/genomes/Drosophila_simulans/dsim_r1.3_FB2010_07/gff)

chr_Dsim	start_Dsim	stop_Dsim	chr_Dmel	start_Dmel	stop_Dmel	Notes
2L	10266499	10268452	X	14805876	14807830	
2L	10270396	10275311	X	14810804	14812777	
2L	10999214	10999699	3L	18245188	18245946	
2L	20232720	20234353	2R	10657104	10658417	
2L	20299846	20300157	X	10326498	10326845	
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2R	3882202	3883050	3L	13081679	13083069	
2R	4621875	4622291	3R	9417998	9418336	
2R	6779351	6781192	3R	8033879	8036079	
2R	8471690	8473688	3L	247495	250314	*confirmed see Table S9.1
2R	8474216	8481704	3L	250229	257950	*confirmed see Table S9.1
2R	8482487	8483090	3L	258350	259097	*confirmed see Table S9.1
2R	8483614	8488356	3L	259286	264378	*confirmed see Table S9.1
2R	8488975	8489561	3L	264782	265599	*confirmed see Table S9.1
2R	8492270	8493159	3L	267764	270340	*confirmed see Table S9.1
2R	8496241	8497365	3L	272210	273370	*confirmed see Table S9.1
2R	8497561	8498894	3L	271640	275021	*confirmed see Table S9.1
2R	8499357	8500565	3L	275164	276661	*confirmed see Table S9.1
2R	8516564	8516848	3L	290727	305292	*confirmed see Table S9.1
2R	8521573	8527566	3L	290727	305292	*confirmed see Table S9.1
2R	8530054	8539812	3L	306207	319880	*confirmed see Table S9.1
2R	8547640	8550369	3L	321232	324717	*confirmed see Table S9.1
2R	8550894	8553274	3L	324985	327987	*confirmed see Table S9.1
2R	8555693	8557587	3L	329822	332306	*confirmed see Table S9.1
2R	8582318	8582686	3L	357981	358486	*confirmed see Table S9.1
2R	8582783	8585414	3L	357851	361383	*confirmed see Table S9.1
2R	8585932	8589521	3L	361478	365681	*confirmed see Table S9.1
2R	8590063	8590554	3L	365664	366437	*confirmed see Table S9.1
2R	8591818	8601097	3L	366524	377316	*confirmed see Table S9.1
2R	8615384	8616176	3L	390941	392824	*confirmed see Table S9.1
2R	8651806	8652279	3L	433230	433891	*confirmed see Table S9.1
2R	8653703	8727410	3L	434258	540589	*confirmed see Table S9.1
2R	8761911	8762932	3L	540851	542147	*confirmed see Table S9.1
2R	8763098	8763730	3L	542122	542916	*confirmed see Table S9.1
2R	8769495	8814396	3L	543070	576065	*confirmed see Table S9.1
3L	4426029	4434446	2L	17501467	17525517	
3L	6733464	6734340	X	17604819	17645728	
3L	9523741	9524778	2R	20136578	20138826	
3L	20381490	20382451	2L	5051193	5053209	
3L	20384075	20386645	2L	5047818	5050877	
3L	20523086	20524147	2L	5718121	5720896	
3R	5290673	5293143	X	20894417	20896915	
3R	5820760	5821392	X	8292973	8297975	
3R	12095492	12096283	4	1144800	1145807	
3R	14631731	14632538	X	19852422	19853421	
X	3126370	3130982	2R	953796	957715	*confirmed see Table S9.1
X	3156780	3159925	2R	978092	982595	*confirmed see Table S9.1
X	3171579	3175408	2R	1004715	1015817	*confirmed see Table S9.1
X	11941312	11941568	3L	19945280	19945890	
X	11943043	11943608	3L	19942282	19944235	
X	16222116	16223702	2R	18691027	18694490	*confirmed see Table S9.1
X	17019891	17022024	3R	5377765	5379469	* possibly confirmed see Table S9.1?