

Supplemental Table S1. Spearman correlations between SNP diversity measures in DGRP Freeze 1.0 (π_{SNP1}) and Freeze 2.0 (π_{SNP2}). Data pairs are estimates for 100 kbp non-overlapping windows along each chromosome arm (*X*, *2L*, *2R*, *3L*, *3R*).

Correlation	Chromosome Arm				
	<i>X</i>	<i>2L</i>	<i>2R</i>	<i>3L</i>	<i>3R</i>
π_{SNP1}, π_{SNP2}	0.941 (2.2e-16)	0.967 (2.2e-16)	0.976 (2.2e-16)	0.982 (2.2e-16)	0.987 (2.2e-16)

Supplemental Table S2. Indel count and size statistics. Indels are polarized with respect to their ancestral state using *D. simulans* as an outgroup.

Chromosome	Total Number	Maximum Size (bp)	Mean Size (bp)	Median Size (bp)	Standard Deviation (bp)
ALL	210,268	2,921	8.47	4	49.54
Deletions	145,015	2,915	8.93	5	18.34
Insertions	65,253	2,921	7.47	3	84.62
2L	46,225				
Deletions	32,138	459	8.71	5	13.30
Insertions	14,087	2,915	6.46	3	66.59
2R	37,479				
Deletions	25,660	2,843	8.96	6	23.02
Insertions	11,819	2,915	7.17	3	78.92
3L	46,749				
Deletions	32,521	2,915	9.15	5	23.67
Insertions	14,228	2,921	7.35	3	82.77
3R	50,600				
Deletions	35,755	616	9.19	6	14.13
Insertions	14,845	2,915	7.41	3	80.77
4	219				
Deletions	171	89	9.10	5	12.32
Insertions	48	2,915	133.54	3	586.99
X	28,996				
Deletions	18,770	783	8.35	5	14.79
Insertions	10,226	2,915	8.88	3	108.27

Supplemental Table S3. Spearman correlation matrix among pair variables. Data pairs are estimates for 100 kb non-overlapping windows along each chromosome arm (*X*, *2L*, *2R*, *3L*, *3R*, *4*). π_{SNP2} refers to nucleotide diversity of SNPs in DGRP Freeze 2.0. Indel refers to insertions and deletions combined, In to insertions only and Del to deletions only. All In and Del calls are for DGRP Freeze 2.0, and are polarized with respect to *D. simulans*. *c* is recombination in cM/Mb. Entries in the table are Spearman's correlation coefficients (*p*-value). n/a: not applicable.

Correlation	Chromosome Arm					
	<i>X</i>	<i>2L</i>	<i>2R</i>	<i>3L</i>	<i>3R</i>	<i>4</i>
π_{SNP2}, π_{indel}	0.660	0.836	0.860	0.886	0.880	0.731
	(2.2e-16)	(2.2e-16)	(2.2e-16)	(2.2e-16)	(2.2e-16)	(6.32e-3)
π_{SNP2}, π_{in}	0.581	0.824	0.842	0.867	0.868	0.687
	(2.2e-16)	(2.2e-16)	(2.2e-16)	(2.2e-16)	(2.2e-16)	(1.20e-2)
π_{SNP2}, π_{del}	0.616	0.756	0.787	0.817	0.795	0.505
	(2.2e-16)	(2.2e-16)	(2.2e-16)	(2.2e-16)	(2.2e-16)	(8.12e-2)
π_{in}, π_{del}	0.613	0.751	0.804	0.798	0.758	0.308
	(2.2e-16)	(2.2e-16)	(2.2e-16)	(2.2e-16)	(2.2e-16)	(3.06e-1)
π_{SNP2}, c	0.387	0.608	0.693	0.707	0.644	n/a
	(2.02e-09)	(2.2e-16)	(2.2e-16)	(2.2e-16)	(2.2e-16)	
π_{indel}, c	0.383	0.504	0.689	0.727	0.571	n/a
	(2.94e-09)	(3.15e-16)	(2.2e-16)	(2.2e-16)	(2.2e-16)	
π_{in}, c	0.376	0.519	0.705	0.748	0.608	n/a
	(6.46e-09)	(2.2e-16)	(2.2e-16)	(2.2e-16)	(2.2e-16)	
π_{del}, c	0.334	0.447	0.619	0.649	0.485	n/a
	(2.99e-07)	(1.11e-12)	(2.2e-16)	(2.2e-16)	(2.2e-16)	

Supplemental Table S4. Functional annotation of segregating variants.

Annotation	Number of Variants
INTRON	2,240,252
INTERGENIC	1,478,922
UPSTREAM	646,329
DOWNSTREAM	575,352
SYNONYMOUS_CODING	351,255
UTR_3_PRIME	204,693
NON_SYNONYMOUS_CODING	182,520
UTR_5_PRIME	118,359
EXON	31,102
START_GAINED	15,992
CODON_DELETION	3,016
FRAME_SHIFT	2,967
CODON_CHANGE_PLUS_CODON_DELETION	1,758
STOP_GAINED	1,532
CODON_CHANGE_PLUS_CODON_INSERTION	1,221
SPLICE_SITE_DONOR	910
SPLICE_SITE_ACCEPTOR	708
CODON_INSERTION	605
SYNONYMOUS_STOP	433
STOP_LOST	301
START_LOST	219
NON_SYNONYMOUS_START	32
UTR_5_DELETED	14
EXON_DELETED	6
UTR_3_DELETED	2
CODON_CHANGE	1

Supplemental Table S5. Effects of inversions and *Wolbachia* infection on quantitative traits. *P*-values from analysis of variance of each trait between inversion genotypes and *Wolbachia* infection status are given.

Train (Number of lines)	Reference	Sex	<i>In(2L)t</i>	<i>In(2R)NS</i>	<i>In(3R)P</i>	<i>In(3R)K</i>	<i>In(3R)Mo</i>	<i>Wolbachia</i>
Starvation resistance (<i>n</i> = 203)	Mackay <i>et al.</i> , 2012	Female	0.776	0.396	0.761	0.025	0.233	0.085
		Male	0.182	0.273	0.281	0.076	0.275	0.723
Startle response (<i>n</i> = 204)	Mackay <i>et al.</i> , 2012	Female	0.216	0.984	0.356	0.968	0.651	0.380
		Male	0.288	0.891	0.565	0.766	0.512	0.285
Chill coma recovery time (<i>n</i> = 176)	Mackay <i>et al.</i> , 2012	Female	0.138	0.337	0.815	0.142	0.544	0.530
		Male	0.309	0.479	0.866	0.700	0.909	0.953
Oxidative stress resistance to paraquat (<i>n</i> = 167)	Weber <i>et al.</i> , 2012	Female	0.856	0.675	0.978	0.302	0.644	0.039
		Male	0.989	0.311	0.248	0.328	0.652	0.022
Oxidative stress resistance to MSB (<i>n</i> = 167)	Weber <i>et al.</i> , 2012	Female	0.308	0.472	0.984	0.660	0.531	0.748
		Male	0.802	0.055	0.367	0.041	0.169	0.006
Sensitivity to oxidative stress (<i>n</i> = 185)	Jordan <i>et al.</i> , 2012	Female	0.463	0.282	0.745	0.548	0.871	0.154
		Male	0.490	0.721	0.821	0.313	0.410	0.578
Night sleep (<i>n</i> = 167)	Harbison <i>et al.</i> , 2013	Female	0.103	0.542	0.193	0.428	0.695	0.945
		Male	0.200	0.204	0.217	0.370	0.977	0.389
Day sleep (<i>n</i> = 167)	Harbison <i>et al.</i> , 2013	Female	0.036	0.301	0.211	0.304	0.277	0.862
		Male	0.024	0.012	0.290	0.650	0.040	0.398
Night bout number (<i>n</i> = 167)	Harbison <i>et al.</i> , 2013	Female	0.021	0.032	0.309	0.472	0.621	0.872
		Male	0.113	0.042	0.906	0.861	0.933	0.077
Day bout number (<i>n</i> = 167)	Harbison <i>et al.</i> , 2013	Female	0.379	0.332	0.643	0.639	0.474	0.911
		Male	0.384	0.020	0.470	0.736	0.004	0.123
Night bout length (<i>n</i> = 167)	Harbison <i>et al.</i> , 2013	Female	0.012	0.135	0.395	0.339	0.430	0.696
		Male	0.180	0.026	0.795	0.914	0.489	0.082
Day bout length (<i>n</i> = 167)	Harbison <i>et al.</i> , 2013	Female	0.104	0.001	0.652	0.407	0.173	0.913
		Male	0.512	0.002	0.788	0.922	<0.0001	0.801
Waking activity (<i>n</i> = 167)	Harbison <i>et al.</i> , 2013	Female	0.588	0.286	0.757	0.114	0.674	0.583
		Male	0.495	0.183	0.870	0.941	0.125	0.961
Olfactory behavior (<i>n</i> = 164)	Swarup <i>et al.</i> , 2013	Female	0.006	0.382	0.128	0.788	0.034	0.947
		Male	0.021	0.411	0.460	0.871	0.038	0.844