Supplemental Data Items

Figure S1. A map of *D. melanogaster* sampling locations in Australia. The sampling sites are indicated by crosses and location information is provided next to the mark.

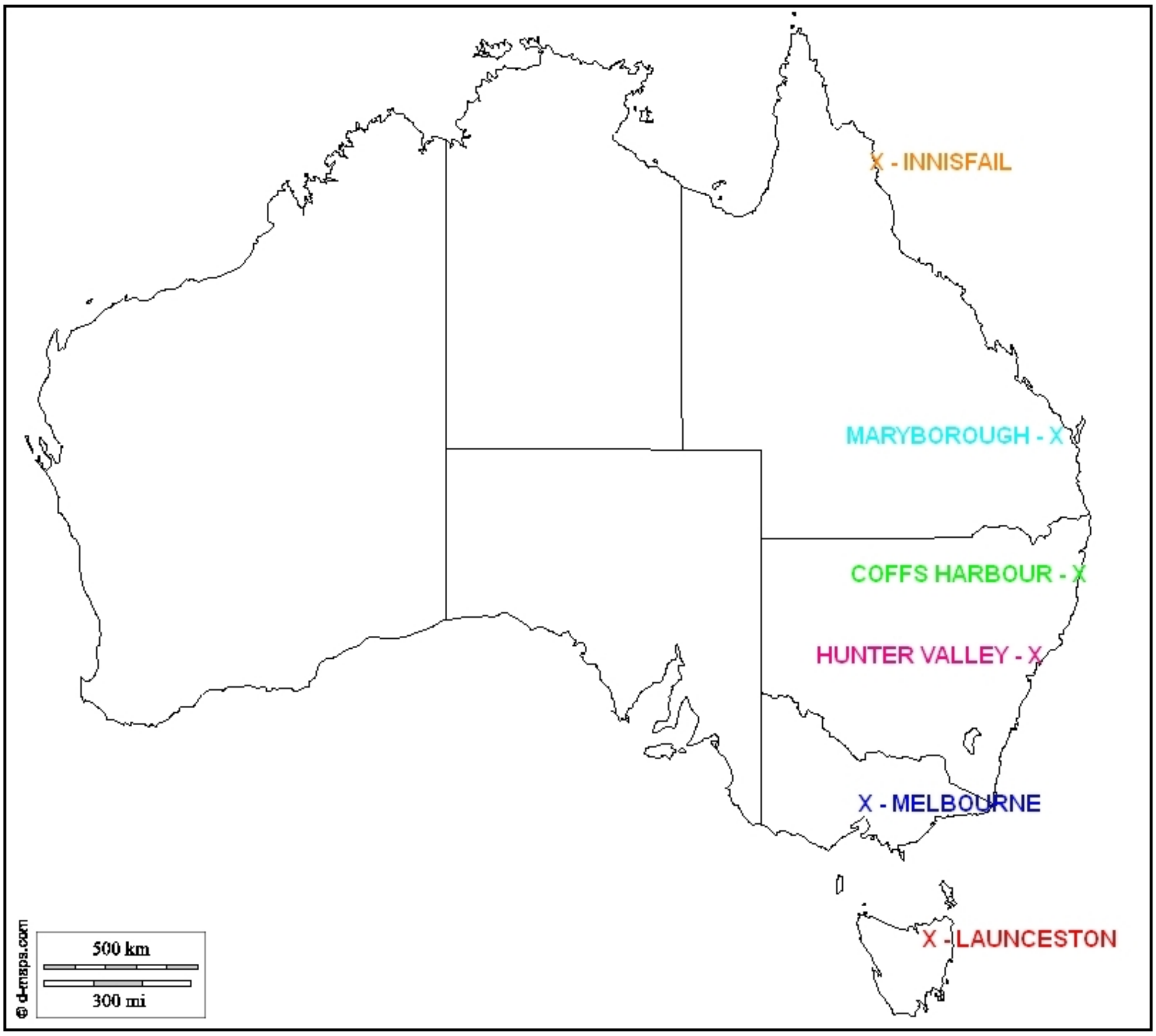

Figure S2. Genetic background of *w*Mel strain used in this study. The alignment of five genes (i.e. *rplF*, *rplB*, *rpoBC*, *fusA*, and *rpsC*) that distinguish *w*Mel and *w*MelCS strains are used in the comparisons. Sequence names were marked with blue (strains used in current study), red (classic *w*Mel strain), and black (*w*MelCS and *w*MelPop strains).

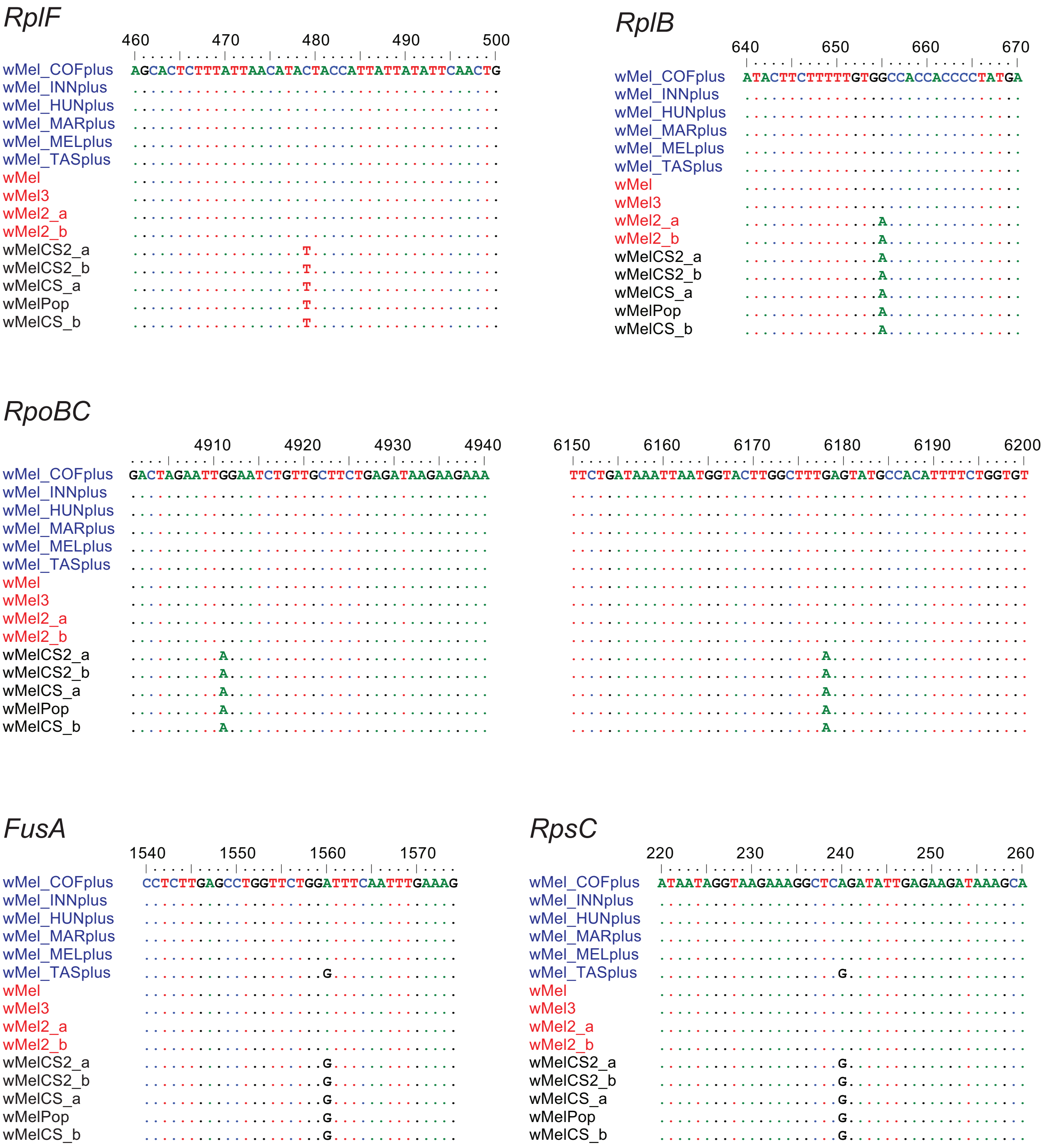

Table S1. Information on each RNA sequencing pool/library.

Pool/Library	Location	No. of individuals	*Wolbachia* status	Filial status	RNA quality (RIN values)	Sequencing output (No. of bases)
INN-	Innisfail	10	negative	F1	8.2	5,095,044,600
INN+	Innisfail	10	*w*Mel positive	F1	9.1	5,216,261,400
MAR-	Maryborough	10	negative	F1	8.6	5,398,528,000
MAR+	Maryborough	10	*w*Mel positive	F1	8.7	5,159,536,000
COF-	Coffs Harbour	10	negative	F1	8.7	4,572,119,600
COF+	Coffs Harbour	10	*w*Mel positive	F1	7.9	5,359,641,200
HUN-	Hunter Valley	10	negative	F1	8.3	5,265,708,400
HUN+	Hunter Valley	10	*w*Mel positive	F1	8.7	4,923,192,400
MEL-	Melbourne	12	negative	F1	8.4	5,286,977,600
MEL+	Melbourne	10	*w*Mel positive	F1	8.3	5,008,758,400
TAS-	Tasmania	10	negative	F3	8.3	4,921,892,800
TAS+	Tasmania	10	*w*Mel positive	F3	7.7	4,851,886,200

Table S2. The presence and abundance of virus, bacteria (*Wolbachia*), and host genes in individual flies (% total non-rRNA reads) (See excel file).

Table S3. Raw Drosophila and *w*Mel Cp density values for samples from 6 different locations in Australia. (See excel file).

Table S4. Comparisons of the mean density of *w*Mel *Wolbachia* and virome abundance for wol+ samples from the different locations.

Location	**Mean***	**N**	**Standard Deviation**	**Virome abundance (% non-ribosomal RNA)**
Coffs Harbour	0.55	10	0.833	0.388
Hunter Valley	1.93	10	1.509	5.392
Innisfail	1.25	10	0.791	8.940
Maryborough	0.47	10	1.460	29.543
Melbourne	2.45	10	1.076	0.708
Tasmania	1.43	10	1.337	0.002
Overall	1.35	60	1.352	0.388

*Computed as the signed difference between Cp values for *w*Mel and control gene

Table S5. Comparisons of *Wolbachia* density and virome abundance in individual *D. melanogaster* from the Melbourne population.

Sample ID	Cp value (Dros RpL40)	Cp value (*w*Mel)	Density value (Dros - *w*Mel Cp)	Virome abundance (% non-ribosomal RNA)
163	26.63	24.15	2.48	3.18
164	26.28	24.94	1.34	1.62
165	27.11	23.9	3.21	82.65
166	26.62	24.23	2.39	51.92
181	26.85	24.06	2.79	44.11
182	26.56	24.12	2.44	0.01
187	28.02	25.79	2.23	12.78
188	26.79	24.97	1.82	6.81
195	26.32	24.18	2.14	26.58
196	26.64	23.79	2.85	21.61
197	26.98	25.02	1.96	0.00
198	27.3	24.34	2.96	0.00
201	26.25	23.63	2.62	0.03
202	26.63	23.95	2.68	0.02
205	26.61	23.02	3.59	1.26
206	27.28	23.18	4.1	0.37
209	26.51	23.81	2.7	0.01
210	26.78	24.56	2.22	0.00
213	26.73	23.35	3.38	0.01
214	27.47	24.83	2.64	0.03