

## Supplemental Information for:

### **Wolbachia causes cytoplasmic incompatibility, but not male-killing in a grain pest beetle**

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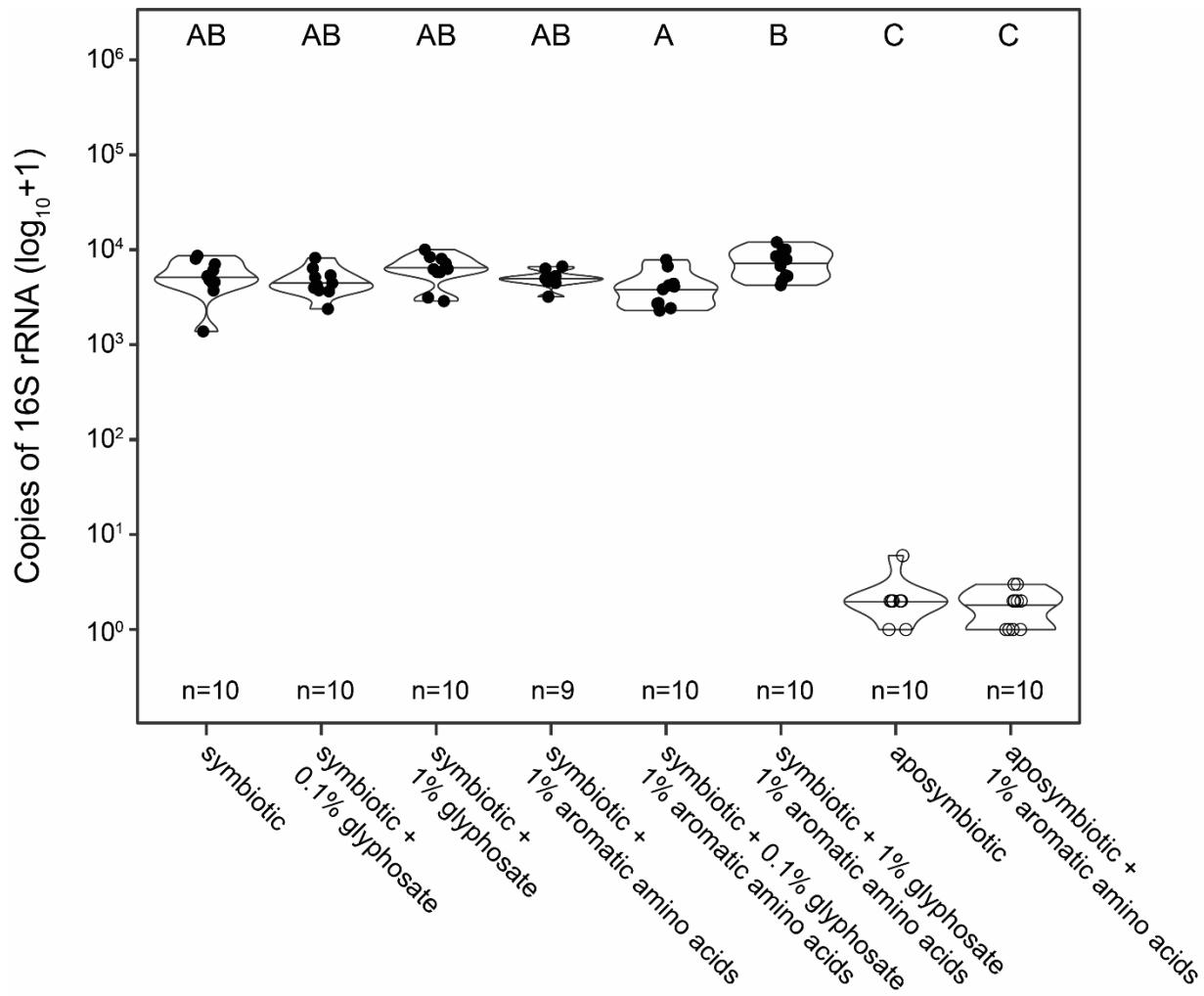
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#### **Table of Contents:**

<b>Suppl. Table 1</b>	Results of Dunn's Test assessing the impact of diet manipulation on <i>wSur</i> titer	Page 2
<b>Suppl. Figure 1</b>	Impact of diet manipulation on <i>wSur</i> titer	Page 3
<b>Suppl. Figure 2</b>	Extended <i>wmk</i> phylogeny	Page 4

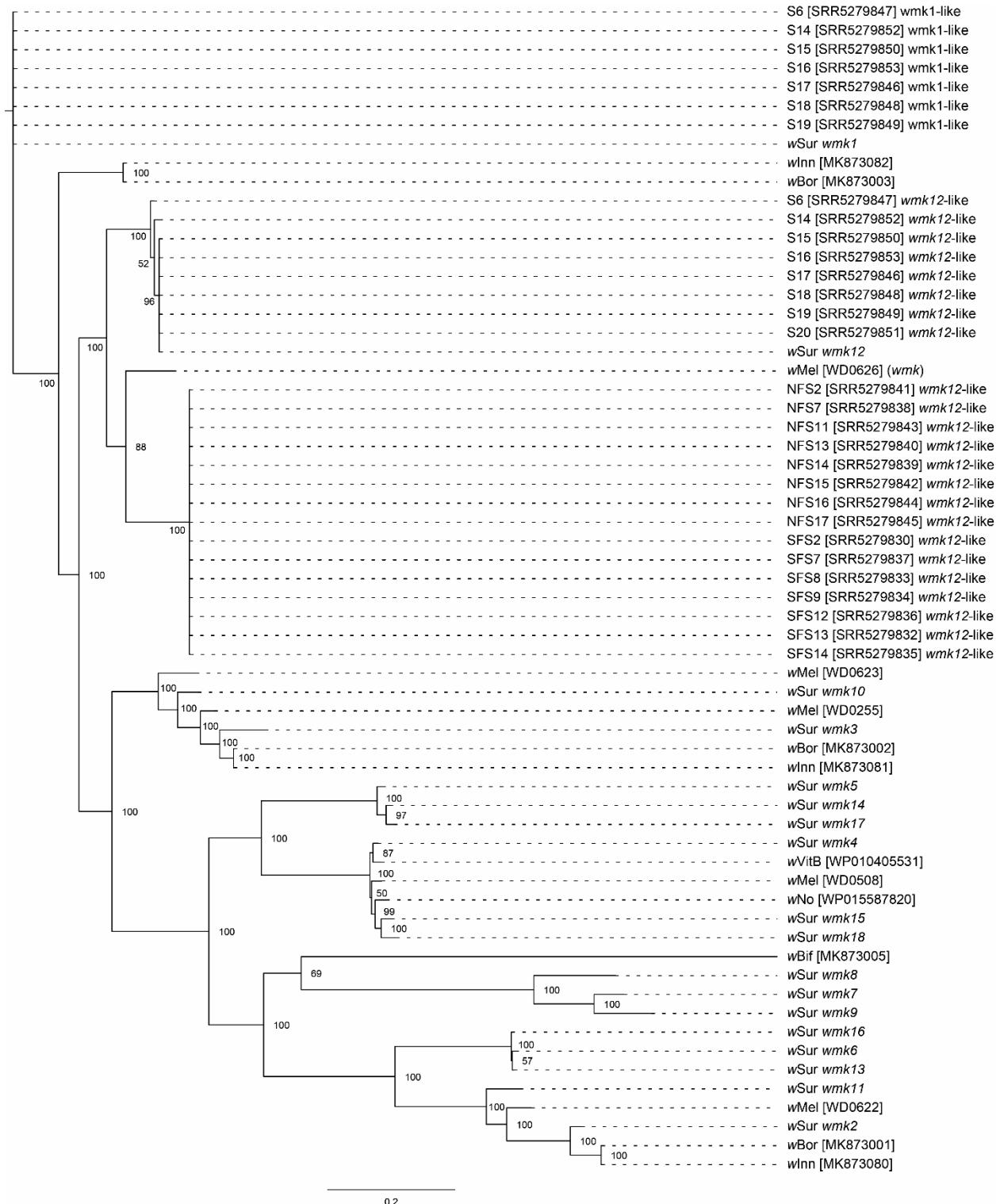
**Supplementary Table 1.** Results of Dunn's Test assessing the impact of glyphosate exposure on wSur titer in one-week-old *O. surinamensis*. Significant results (P.adj < 0.05) are highlighted in bold. Apo = aposymbiotic, Sym = symbiotic, 1% AA = addition of 1% (w/w) of each aromatic amino acid (tyrosine, phenylalanine, tryptophan), 0.1 / 1% G = addition of 1% (w/w) glyphosate

Comparison	Z	p	Adjusted p
Apo - Apo + 1 % AA	1.17E-01	9.07E-01	9.07E-01
<b>Apo - Sym</b>	<b>-3.85E+00</b>	<b>1.16E-04</b>	<b>5.41E-04</b>
<b>Apo + 1 % AA - Sym</b>	<b>-3.97E+00</b>	<b>7.13E-05</b>	<b>3.99E-04</b>
<b>Apo - Sym + 0.1 % G</b>	<b>-3.17E+00</b>	<b>1.52E-03</b>	<b>4.24E-03</b>
<b>Apo + 1 % AA - Sym + 0.1 % G</b>	<b>-3.29E+00</b>	<b>1.01E-03</b>	<b>3.13E-03</b>
Sym - Sym + 0.1 % G	6.83E-01	4.95E-01	6.29E-01
<b>Apo - Sym + 1 % G</b>	<b>-4.51E+00</b>	<b>6.53E-06</b>	<b>4.57E-05</b>
<b>Apo + 1 % AA - Sym + 1 % G</b>	<b>-4.63E+00</b>	<b>3.73E-06</b>	<b>3.49E-05</b>
Sym - Sym + 1 % G	-6.54E-01	5.13E-01	6.25E-01
Sym + 0.1 % G - Sym + 1 % G	-1.34E+00	1.81E-01	2.99E-01
<b>Apo - Sym + 1 % AA</b>	<b>-3.52E+00</b>	<b>4.39E-04</b>	<b>1.54E-03</b>
<b>Apo + 1 % AA - Sym + 1 % AA</b>	<b>-3.63E+00</b>	<b>2.84E-04</b>	<b>1.14E-03</b>
Sym - Sym + 1 % AA	2.36E-01	8.13E-01	8.43E-01
Sym + 0.1 % G - Sym + 1 % AA	-4.28E-01	6.68E-01	7.20E-01
Sym + 1 % G - Sym + 1 % AA	8.73E-01	3.83E-01	5.10E-01
<b>Apo - Sym + 1 % AA + 0.1 % G</b>	<b>-2.55E+00</b>	<b>1.09E-02</b>	<b>2.53E-02</b>
<b>Apo + 1 % AA - Sym + 1 % AA + 0.1 % G</b>	<b>-2.66E+00</b>	<b>7.72E-03</b>	<b>1.96E-02</b>
Sym - Sym + 1 % AA + 0.1 % G	1.31E+00	1.91E-01	2.97E-01
Sym + 0.1 % G - Sym + 1 % AA + 0.1 % G	6.25E-01	5.32E-01	6.21E-01
Sym + 1 % G - Sym + 1 % AA + 0.1 % G	1.96E+00	4.98E-02	9.96E-02
Sym + 1 % AA - Sym + 1 % AA + 0.1 % G	1.04E+00	3.00E-01	4.20E-01
<b>Apo - Sym + 1 % AA + 1 % G</b>	<b>-5.06E+00</b>	<b>4.09E-07</b>	<b>5.72E-06</b>
<b>Apo + 1 % AA - Sym + 1 % AA + 1 % G</b>	<b>-5.18E+00</b>	<b>2.20E-07</b>	<b>6.15E-06</b>
Sym - Sym + 1 % AA + 1 % G	-1.21E+00	2.26E-01	3.33E-01
Sym + 0.1 % G - Sym + 1 % AA + 1 % G	-1.89E+00	5.83E-02	1.09E-01
Sym + 1 % G - Sym + 1 % AA + 1 % G	-5.56E-01	5.78E-01	6.47E-01
Sym + 1 % AA - Sym + 1 % AA + 1 % G	-1.41E+00	1.57E-01	2.75E-01
Sym + 1 % AA + 0.1 % G - Sym + 1 % AA + 1 % G	-2.52E+00	1.18E-02	2.54E-02



**Supplement Figure 1:** Titer of wSur in *O. surinamensis* adults reared on different food compositions. The data distribution is visualized with violin plots and an additional horizontal line depicting the median. Filled circles represent specific target amplification, empty circles off-target amplification during late qPCR cycles, identified by melting curve analysis. Different letters indicate significant differences between experimental treatments (Dunn's Test, adjusted  $p \leq 0.05$ ).

# MOLECULAR ECOLOGY



**Supplement Figure 2 (previous page):** Phylogeny of *wmk* homologues including *wmk1* and *wmk12*-like homologues from *O. surinamensis* sequencing libraries from different collection sites in Israel, including two feral field populations (SFS, SRR-52779830 – SRR5279837 and NFS, SRR5279838 – SRR5279845) and a storage facilities population (S, SRR5279846 - SRR5279853). *wmk1*-like homologues only occurred in wSur from *O. surinamensis* JKI and Israeli storage populations (S). *wmk12*-like homologues from storage populations (S) clustered strictly with wSur *wmk12* and exhibited the same frame shift mutation as *wmk12* in wSur from the *O. surinamensis* JKI population, while the respective homologues clustered separately and do encode a complete protein analogous to *wmk* from wMel.