**Supplemental Information for:**

**Recent infection by *Wolbachia* alters microbial communities in wild *Laodelphax striatellus* populations**

Xing-Zhi Duan1†, Jing-Tao Sun1†, Lin-Ting Wang1, Xiao-Han Shu1, Matsukura Keiichiro2, Yan Guo1, Yu-Xi Zhu1, Xiao-Li Bing1, Ary A. Hoffmann3, Xiao-Yue Hong1\*

1Department of Entomology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China  
2NARO Kyushu Okinawa Agricultural Research Center, 2421 Suya, Koshi, Kumamoto 861-1192, Japan

3School of BioSciences, Bio21 Institute, The University of Melbourne, Victoria 3010, Australia

**Table S1** Summary of collection details. The population code (ID), province, city, county, latitude, longitude, and date of the field collections assessed here are provided.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ID | Province | City | County | latitude | longitude | Date |
| HRB | Heilongjiang | Ha'erbin | Yanshou | 45.48°N | 126.39°E | 08/14/2017 |
| MDJ | Heilongjiang | Mudanjiang |  | 44.55°N | 129.64°E | 07/05/2012 |
| DD | liaoning | Dandong |  | 40.17°N | 124.39°E | 07/16/2012 |
| DZ | Shandong | Dezhou |  | 37.43°N | 116.24°E | 06/22/2012 |
| XX | Henan | Xinxiang |  | 35.32°N | 113.97°E | 06/22/2010 |
| YC | Jiangsu | Yancheng | Xiangshui | 34.11°N | 119.76°E | 06/13/2017 |
| HN | Anhui | Huainan | Fengtai | 32.72°N | 116.67°E | 07/23/2012 |
| XY | Henan | Xinyang |  | 32.16°N | 114.09°E | 06/25/2010 |
| NJ | Jiangsu | Nanjing |  | 32.04°N | 118.87°E | 06/10/2010 |
| LY | Fujian | Longyan |  | 25.12°N | 117.02°E | 05/27/2010 |
| GZ | Guangdong | Guangzhou |  | 23.15°N | 113.35°E | 06/11/2010 |
| SAP | Hokkaido | Sapporo | Hokkaido | 43.03°N | 141.44°E | 08/30/2017 |
| JOE | Hokuriku | Joetsu | Niigata | 37.12°N | 138.27°E | 09/02/2017 |
| KUM | Kanto | Kumagaya | Saitama | 36.17°N | 139.35°E | 09/02/2017 |
| OHD | Chugoku | Ohda | Shimane | 35.15°N | 132.40°E | 07/26/2017 |
| YUK | Kyushu | Yukuhashi | Fukuoka | 33.71°N | 130.95°E | 05/29/2017 |
| ICH | Kyushu | Ichikikushikino | Kagoshima | 31.75°N | 130.25°E | 05/29/2017 |

**Table S2** Specific primers used for PCR in this study.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Organism | Target | Primer name | Primer sequence (5’→3’) | Product  Size (bp) | Annealing  temp (°C) | Reference |
| SBPH | *COI* | COIF  COIR | TCTCATTACATATCGCTGGAGTTAG  GTAGTCTGAATATCGTCGTGGTATT | 887 | 55 | Designed in this study |
| *Wolbachia* | wsp | wsp-F  wsp-R | TGGTCCAATAAGTGATGAAGAAAC  AAAAATTAAACGCTACTCCA | 599 | 52 | Zhao *et al*. [38] |

**Table S3** Pairwise *F*ST estimates between populations based on a sequence of the mitochondrial *COI* gene. Population codes are given in Table S1.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | HRB | MDJ | DD | DZ | XX | YC | HN | XY | NJ | LY | GZ | JOE | KUM | OHD | YUK |
| MDJ | 0.003 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| DD | **0.182** | **0.250** |  |  |  |  |  |  | |  |  |  |  |  |  |
| DZ | **0.130** | **0.194** | -0.022 |  |  |  |  |  | |  |  |  |  |  |  |
| XX | 0.024 | **0.071** | 0.030 | 0.001 |  |  |  |  |  |  |  |  |  |  |  |
| YC | **0.344** | **0.403** | 0.038 | **0.051** | **0.155** |  |  |  |  |  |  |  |  |  |  |
| HN | **0.185** | **0.260** | -0.020 | -0.023 | 0.029 | **0.036** |  |  |  |  |  |  |  |  |  |
| XY | **0.215** | **0.284** | -0.007 | -0.018 | **0.052** | 0.021 | -0.014 |  |  |  |  |  |  |  |  |
| NJ | **0.061** | **0.125** | 0.013 | -0.012 | -0.022 | **0.131** | 0.005 | **0.032** |  |  |  |  |  |  |  |
| LY | **0.151** | **0.218** | 0.002 | -0.022 | 0.015 | **0.058** | -0.010 | -0.024 | 0.005 |  |  |  |  |  |  |
| GZ | **0.390** | **0.457** | 0.034 | **0.059** | **0.175** | -0.019 | 0.038 | 0.026 | **0.151** | **0.072** |  |  |  |  |  |
| JOE | **0.211** | **0.269** | -0.024 | -0.006 | 0.048 | **0.030** | -0.003 | 0.009 | **0.041** | 0.021 | 0.031 |  |  |  |  |
| KUM | **0.511** | **0.568** | **0.130** | **0.165** | **0.287** | 0.020 | **0.140** | **0.129** | **0.271** | **0.186** | 0.009 | **0.106** |  |  |  |
| OHD | **0.269** | **0.332** | 0.012 | 0.005 | **0.089** | 0.024 | 0.026 | -0.004 | **0.064** | 0.021 | **0.043** | 0.020 | **0.141** |  |  |
| YUK | **0.110** | **0.181** | -0.008 | -0.012 | 0.002 | **0.105** | 0.003 | 0.028 | -0.015 | 0.017 | **0.117** | 0.019 | **0.235** | **0.050** |  |
| ICH | **0.422** | **0.486** | **0.066** | **0.083** | **0.208** | -0.022 | 0.070 | 0.044 | **0.182** | **0.096** | -0.023 | **0.061** | 0.011 | **0.041** | **0.150** |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

The bold indicated significant indices (*P* <0.05).

**Table S4** Annual mean temperatures (Bio1) and the annual mean precipitation (Bio12) of the 17 locations obtained from DIVA-GIS 7.5.0.

|  |  |  |
| --- | --- | --- |
| ID | Bio1 | Bio12 |
| HRB | 3.508 | 565 |
| MDJ | 4.321 | 545 |
| DD | 8.871 | 1040 |
| DZ | 13.863 | 505 |
| XX | 14.963 | 585 |
| YC | 13.946 | 773 |
| HN | 15.754 | 799 |
| XY | 15.429 | 1089 |
| NJ | 15.625 | 1017 |
| LY | 20.633 | 1681 |
| GZ | 22.363 | 1739 |
| SAP | 7.875 | 1140 |
| JOE | 13.458 | 2572 |
| KUM | 14.496 | 1273 |
| OHD | 14.396 | 1784 |
| YUK | 15.842 | 1709 |
| ICH | 16.846 | 2388 |

**Table S6** Relative abundance of bacterial 16S rRNA genes at the genus level observed for Chinese, Japanese and all populations.

|  |  |  |  |
| --- | --- | --- | --- |
| Genus classes | China (%) | Japan (%) | All samples (%) |
| *Wolbachia* | 87.9 | 66.4 | 81.2 |
| *Spiroplasma* | 3.55 | 5.00 | 4.01 |
| *Diplorickettsia* | 0.00 | 10.9 | 3.41 |
| *Asaia* | 2.47 | 5.56 | 3.44 |
| *Pantoea* | 1.04 | 2.08 | 1.36 |
| *Herbaspirillum* | 1.03 | 1.05 | 1.03 |
| *unclassified\_f\_\_Enterobacteriaceae* | 0.88 | 1.29 | 1.01 |
| *Microbacterium* | 0.81 | 1.23 | 0.94 |
| *Acinetobacter* | 0.10 | 2.33 | 0.80 |
| *unclassified\_f\_\_Xanthomonadaceae* | 0.09 | 1.06 | 0.39 |
| others | 2.13 | 3.13 | 2.41 |

**Table S7** Effects of factors in the structural equation model (SEM) analysis undertaken on 16 populations where *Wolbachia* was fixed in the population.

Effects of the latitude on *F*st, Bio1 and Bio12:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variation | Effect | Effect size ± SE | Z value | *P* |
| Genetic differentiation (*F*st) | Latitude | 0.387 ± 0.084 | 4.583 | <0.001 |
| Annual mean temperature (Bio1) | 0.929 ± 0.033 | 27.800 | <0.001 |
| Annual precipitation (Bio12) | 0.001 ± 0.091 | 0.010 | 0.992 |

Effects of the longitude on *F*st, Bio1 and Bio12:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variation | Effect | Effect size ± SE | Z value | *P* |
| Genetic differentiation (*F*st) | Longitude | 0.029 ± 0.084 | 0.347 | 0.728 |
| Annual mean temperature (Bio1) | -0.053 ± 0.033 | -1.590 | 0.112 |
| Annual precipitation (Bio12) | 0.156 ± 0.091 | 1.720 | 0.085 |

Effects of *F*st, Bio1, Bio12, Lat and Lon on the microbial Bray Curtis dissimilarity (MCSD):

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variation | Effect | Effect size ± SE | Z value | *P* |
| MCSD | Genetic differentiation (*F*st) | 0.257 ± 0.079 | 3.268 | <0.001 |
| Latitude | -0.115 ± 0.201 | -0.573 | 0.567 |
| Longitude | 0.418 ± 0.074 | 5.643 | <0.001 |
| Annual mean temperature (Bio1) | -0.166 ± 0.199 | -0.837 | 0.402 |
| Annual precipitation (Bio12) | 0.246 ± 0.073 | 3.358 | <0.001 |

Effects of *F*st, Bio1, Bio12, Lat and Lon on the KL divergence (KLD):

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variation | Effect | Effect size ± SE | Z value | *P* |
| KLD | Genetic differentiation (*F*st) | 0.230 ± 0.097 | 2.378 | 0.017 |
| Latitude | 0.014 ± 0.247 | 0.055 | 0.956 |
| Longitude | -0.055 ± 0.091 | -0.597 | 0.550 |
| Annual mean temperature (Bio1) | -0.204 ± 0.245 | -0.833 | 0.405 |
| Annual precipitation (Bio12) | -0.108 ± 0.090 | -1.195 | 0.232 |

**Table S10** After *Wolbachia* was excluded from the *Wolbachia*-infected adults, the [composition](javascript:;)

of all samples from the SAP population.

|  |  |
| --- | --- |
| Sample Name | Sequences |
| w+1 | 16701 |
| w+2 | 5246 |
| w+3 | 4625 |
| w+4 | 1573 |
| w+5 | 4595 |
| w+6 | 1144 |
| w+7 | 7186 |
| w+8 | 6852 |
| w+9 | 3671 |
| w+10 | 17983 |
| w-1 | 51721 |
| w-2 | 51168 |
| w-3 | 63157 |
| w-4 | 59070 |
| w-5 | 62467 |
| w-6 | 64472 |
| w-7 | 60195 |
| w-8 | 59871 |
| w-9 | 62564 |

**Table S11** Relative abundance of bacterial 16S rRNA genes at the genus level observed for *Wolbachia*-infected females (after removal of *Wolbachia* reads), *Wolbachia*-uninfected females and all samples.

|  |  |  |  |
| --- | --- | --- | --- |
| Genus classes | W+ (%) | W- (%) | All samples (%) |
| *Thermus* | 26.5 | 5.58 | 16.6 |
| *Spiroplasma* | 21.6 | 0.84 | 11.8 |
| *Arsenophonus* | 7.76 | 13.4 | 10.5 |
| *Ralstonia* | 17.9 | 0.91 | 9.84 |
| *Prevotella\_9* | 0.04 | 15.1 | 7.18 |
| *Prevotellaceae\_NK3B31\_group* | 0.02 | 5.58 | 2.65 |
| *Lactobacillus* | 1.16 | 3.93 | 2.47 |
| *norank\_f\_\_Muribaculaceae* | 2.48 | 1.81 | 2.16 |
| *unclassified\_f\_\_Lachnospiraceae* | 0.37 | 4.04 | 2.11 |
| *Megasphaera* | 0.00 | 4.17 | 1.97 |
| *Dialister* | 0.00 | 2.98 | 1.41 |
| *Streptococcus* | 0.09 | 1.98 | 0.98 |
| *Succinivibrio* | 0.00 | 1.68 | 0.80 |
| *Prevotella\_2* | 0.00 | 1.30 | 0.61 |
| *Clostridium\_sensu\_stricto\_1* | 0.09 | 1.19 | 0.61 |
| *Selenomonas* | 0.00 | 1.07 | 0.51 |
| *norank\_f\_\_Veillonellaceae* | 0.00 | 0.94 | 0.45 |
| *norank\_f\_\_AKYH767* | 0.69 | 0.00 | 0.35 |
| others | 21.3 | 33.5 | 27.0 |

**Table S12** Measures of species richness and evenness of SBPH from 10 *Wolbachia*-infected females (excluding *Wolbachia* reads) and 9 *Wolbachia*-free females from the SAP population.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Samples | Sobs | Shannon | Simpson | Ace | Chao | Coverage |
| w+1 | 223 | 3.356 | 0.190 | 723.031 | 402.143 | 0.872 |
| w+2 | 224 | 4.508 | 0.025 | 457.459 | 397.906 | 0.898 |
| w+3 | 34 | 0.667 | 0.760 | 267.682 | 94.000 | 0.976 |
| w+4 | 159 | 3.419 | 0.108 | 230.706 | 202.826 | 0.938 |
| w+5 | 85 | 1.516 | 0.516 | 174.559 | 164.688 | 0.951 |
| w+6 | 121 | 2.611 | 0.176 | 216.559 | 208.652 | 0.938 |
| w+7 | 41 | 1.380 | 0.372 | 92.198 | 101.000 | 0.976 |
| w+8 | 42 | 0.894 | 0.682 | 192.273 | 112.200 | 0.974 |
| w+9 | 56 | 1.496 | 0.414 | 122.637 | 79.400 | 0.974 |
| w+10 | 26 | 0.787 | 0.627 | 175.754 | 77.000 | 0.983 |
| w-1 | 38 | 1.267 | 0.444 | 80.397 | 74.143 | 0.978 |
| w-2 | 54 | 1.347 | 0.414 | 162.009 | 140.667 | 0.961 |
| w-3 | 142 | 3.828 | 0.043 | 204.044 | 203.600 | 0.946 |
| w-4 | 244 | 4.860 | 0.013 | 343.976 | 341.133 | 0.909 |
| w-5 | 161 | 3.805 | 0.052 | 366.133 | 261.100 | 0.925 |
| w-6 | 172 | 3.867 | 0.049 | 365.448 | 284.452 | 0.919 |
| w-7 | 228 | 4.495 | 0.026 | 499.878 | 373.357 | 0.893 |
| w-8 | 154 | 3.725 | 0.053 | 334.910 | 250.464 | 0.929 |
| w-9 | 149 | 3.652 | 0.066 | 249.539 | 270.714 | 0.931 |