

SUPPLEMENTARY DATA

Fluorescence *in situ* hybridization (FISH). FISH was performed as previously described (3). Briefly, adult were fixed overnight in Carnoy's fixative (chloroform:ethanol:glacial acetic acid, 6:3:1, v/v), then decolorized in 6% H₂O₂ in ethanol for 2 h and hybridized overnight in hybridization buffer (20 mM Tris-HCl pH 8.0, 0.9 M NaCl, 0.01% w/v sodium dodecyl sulfate, 30% v/v formamide) containing 10 pmol fluorescent probes per ml.. For specific targeting of *Portiera*, BTP1-Cy3 (5'-Cy3-TGTCAGTGTCCAGCCAGGAAG-3') probe was used. The stained samples were mounted whole in hybridization buffer and viewed under an IX81 Olympus FluoView500 confocal microscope. Specificity of detection was confirmed using no-probe and *Rickettsia*-free whitefly controls.

Transmission Electron microscopy (TEM). Detached adult whitefly abdomens were fixed overnight in 2.5% glutaraldehyde in 1X PBS at 4 °C and processed using a standard method for TEM: rinsing in 1X PBS buffer, osmification, another rinse in 1X PBS buffer, dehydration in an ascending ethanol series, acetone incubation and embedding in epoxy resin Agar 100 (Agar Scientific, Essex, England). Thin sections (60–90 nm) were cut using an ultramicrotome, stained with aqueous uranyl acetate and lead citrate and examined in a Tecnai 12 electron microscope (Philips/FEI, Eindhoven, The Netherlands).

DNA isolation and sequencing. High-quality DNA (>40kb) was extracted from whole bodies of 150 pooled insect adults of each species using the CTAB method. Paired-end libraries with 180-bp-insertions and 5-kb mate pair libraries were constructed and sequenced on HiSeq 2000 at 2 × 100 cycles. Adaptors and low quality reads were removed before assembly. ALLPATH-LG (2) and Velvet (5) were used for *de novo* assembly. Based on the depth of coverage, paired-end connections (180 ± 10 bp) and 5kb mate-pair connection, *Portiera* contigs were selected and linked into a closed circular

molecule by customized scripts. As there are thousands of *Portiera* copies in each individual and 150 individuals from each biotype were pooled, our *Portiera* contigs represent sequences of a *Portiera* population for each biotype. We observed a limited number of short repeats/homopolymers that appear polymorphic, and have taken the major allele in these cases as the consensus sequence. Over 1 million reads were mapped to each of the final assemblies by SMALT (<http://www.sanger.ac.uk/resources/software/smalt/>; map quality 40, base quality 10). Forward and reverse reads were merged before mapping as they were made intentionally to have 20 bp overlap to pass through possible short-repetitive sequence regions. Assembly accuracy and the coverage (at least $25 \times$ coverage for each nucleotide) were manually curated. 10 kb (33284-34545; 272425-280261; location is based on *Portiera* WB genome) were successfully amplified by using primer pairs based on the assembled genome and further validated by Sanger sequencing. Annotation was performed on the RAST annotation server (4), with automatic frameshift-fixing and followed by manual inspection of all gene predictions.

Comparative genomic analyses. The two *Portiera* genomes were aligned by Mauve using default settings (1). As stated above, we took major alleles during the assembly. Thus, some of these divergent regions might only reflect the allele frequency in two *Portiera* populations. In order to explore the unique regions for each *Portiera*, we mapped all the reads from one whitefly strain to the *Portiera* genome assembly of the other strain using SMALT under the same parameters (map quality 40, base quality 10). The regions of each reference genome that lack any aligned reads derived from the other strain were identified as unique regions.

LEGENDS FOR SUPPLEMENTARY FIGURES AND TABLES

FIG S1 Functional gene content in the *Portiera* genome by COG categories.

Assignments were based on RAST (see text for more details).

Table S1a Annotation of assembled coding sequences from the *Portiera* WQ genome, including the location for each coding sequence, strand orientation, functional annotation, nucleotide and amino acid sequences.

Table S1b Annotation of assembled coding sequences from the *Portiera* WB genome, including the location for each coding sequence, strand orientation, functional annotation, nucleotide and amino acid sequences.

Table S1c Comparative functional annotation of assembled coding sequences from the *Portiera* WB and WQ genomes, including the location for each coding sequence and strand orientation.

Table S2 Discovery of polymorphic sites in the *Portiera* WQ genome.

Table S3 Unique regions identified in the *Portiera* WB and *Portiera* WQ genomes in the vicinity of coding regions or within intergenic regions.

Table S4. Unique regions in *Portiera* WB genome within or near (100bp) gene-coding regions.

Table S5. Unique regions in *Portiera* WQ genome within or near (100bp) gene-coding regions.

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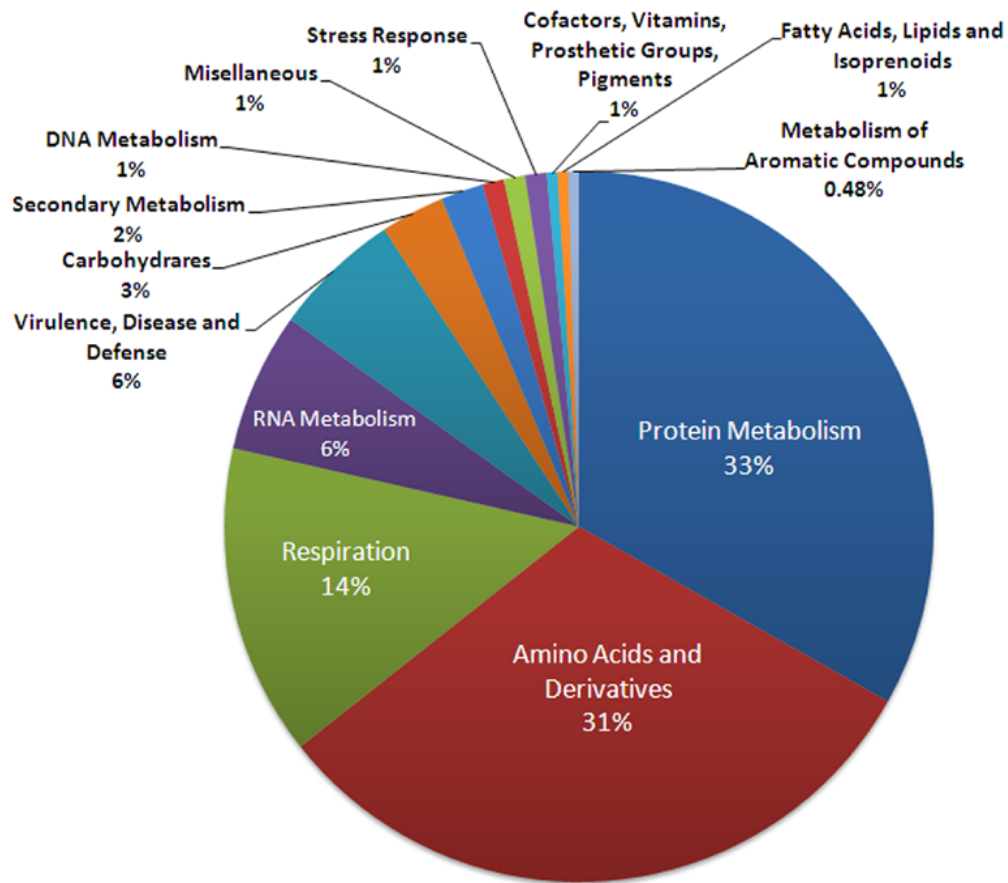


FIG S1 Functional gene content in the *Portiera* genome by COG categories. Assignments were based on RAST (see text for more details).