**Supplementary Materials and Methods**

**Aphid lines**

The LSR1 *A. pisum* strain was used for all aphid host-plant trials in this study (*1*). For host-plant trials the LSR1 strain was divided into six independent sub-lines: three on fava (F1, F2, and F3) and three on alfalfa (A1, A2, and A3). Aphid sub-lines were reared at the same conditions as described in Hansen and Moran (*2*). Before the start of all trials, the three fava sub-lines were reared on the same developmental stage of fava bean (F1, F2, F3; fava bean= 23 ± 2 days after germination (~ 5 whorls)) for over 10 generations, and the three alfalfa sub-lines were reared on the same developmental stage of alfalfa (A1, A2, A3; alfalfa= 44 ± 2 days after germination) for over 10 generations. These plant developmental stages were chosen because they correspond with plant developmental ages used previously in pea aphid free amino acid stylectomy trials, where amino acid profiles varied significantly between fava bean and alfalfa sap (*3*).

**Aphid mass assessment**

Weights of asexual adults (1 day after final instar molt) were measured using a CAHN 29 automatic electrobalance (Cahn, Cerritos, CA) for 20 individuals per sub-line (F1, F2, F3, A1, A2, A3). For statistical analyses, General Linear Models (GLM) was used treating aphid mass as a dependent variable and host plant treatment as a fixed factor with insect line nested within host plant treatment using IBM SPSS Statistics version 23 (IBM SPSS, Armonk, NY). Tukey’s multiple comparison post-hoc tests were used to determine aphid mass differences between sub-lines.

**Determination of *Buchnera* cell abundance using RT-qPCR**

After weight measurements (see above) parthenogenic female adults were preserved in 95% ETOH for *Buchnera* cell abundance measurements using Real Time quantitative PCR (RT-qPCR). Also, several offspring (1st day nymphs) from these adults were reared on plants until the first day of reproduction (pre-reproductive phase of alfalfa aphids = ~10-11 days and 9-10 days for fava aphids), and they were collected from each sub-line and preserved for *Buchnera* cell abundance measurements. DNA of individual aphids was extracted with the Qiagen DNeasy DNA extraction kit following the manufacture’s protocol (Valencia, CA, USA) (six individuals per life stage per sub-line were extracted; N=72 individuals total). RT-qPCR was conducted on each aphid individual using the Eppendorf Mastercycler epgradient realplex2 (Hamburg, Germany). For RT-qPCR reactions KAPA SYBR FAST universal qPCR kit (Wilmington, MA) was used for concentrations and cycle conditions (2-step qPCR). A standard curve was created for each reaction plate by diluting target gene plasmids for both a single copy gene in *Buchnera* (ATP synthase F0F1 subunit C, *atpE*) and the pea aphid (*Acyrthosiphon pisum* 60 kDa heat shock protein (LOC100168563), *HS60*). Primer sequences for these *Buchnera* and aphid genes respectively are: BAp\_atpE\_F1- 5' -CCG CTA GGC AAC CTG ATT TA-3’; BAp\_atpE\_F1- 5' -CAA TCA TTG GAA TCG CAT CA-3’; and Aphid HS60 F1- 5'- GCC AAG AAG GTA ATG AAC TG-3’; Aphid HS60 R1- 5'- TCA ACA GCA AAG TGT CAT C-3’. The standard curve method for relative quantification (*4*) was used to compute the normalized *Buchnera* expression value for each aphid sample (i.e. normalized to the aphid gene HS60). For statistical analyses, General Linear Models (GLM) was used treating the normalized *Buchnera* expression value as the dependent variable and host plant treatment and life stage (1st instar nymph and adult) as fixed factors, with insect line nested within host plant treatment using IBM SPSS Statistics version 23 (IBM SPSS, Armonk, NY).

**TEM imaging of *Buchnera* cells**

For transmission electron microscopy (TEM) images, aphids on the last nymphal stage (4th instar) were dissected in buffer and fixed for 2 hours. The tissue was dehydrated in ethanol for 12 hours. Subsequently, bacteriocytes for each host plant treatment were observed using a TEM microscope. For each TEM image, the number of *Buchnera* cells were counted within a unit area (100 µm2) using Cell Counter plugin of ImageJ version 1.8.0 (*5*). The means of each bacteriocyte image (N=7 for fava; N=5 for alfalfa) were compared using a two-sample t-test.

**Determination ofbacteriocyte abundance**

For both host plant treatments, at least 12 aphids from each sub-line were dissected and bacteriocytes were counted using a light microscope (N=45 and 46 aphid individuals for fava and alfalfa treatments, respectively). For statistical analyses GLM was used treating bacteriocyte count as the dependent variable and host plant treatment as a fixed factor, with insect line nested within host plant treatment using IBM SPSS Statistics version 23 (IBM SPSS, Armonk, NY).

**RNA-Seq analysis**

For RNA-Seq trials, the same six sub-lines (F1, F2, F3, A1, A2, A3) were analyzed.Aphid bacteriocyte and body tissue dissections were conducted as in Hansen and Moran (*2*) during the aphid's fourth instar, giving 12 samples: bacteriocytes of alfalfa feeding aphids (ABAC), body cells of alfalfa feeding aphids (ABODY), bacteriocytes of fava feeding aphids (FBAC), and body cells of fava feeding aphids (FBODY), each with three biological samples. For dissections, one aphid sub-line was dissected at a time for a two-week duration to pool enough material for RNA extractions. All sub-lines were randomly dissected one after the other over a continuous time block of three months, which allowed us to pool approximately 100 aphids to get enough RNA concentration. For each aphid, bacteriocytes were dissected out and collected. Body cell samples were prepared from the remainder of the dissected tissue by removing any remaining bacteriocytes and embryos. RNA extractions were conducted similar to Hansen and Moran (*2*), except RNA > 200 bp was retained for sequencing using Illumina HiSeq 2500 (Illumina, San Diego, CA).

Illumina library preparation and sequencing were conducted by Yale University’s Keck Genome Sequencing Center. Libraries were sequenced as paired-end 76-mers using Illumina’s pipeline. Three samples were sequenced per lane. Reads for all RNA-Seq samples (12 total: 6 bacteriocyte and 6 corresponding body tissue samples) were submitted to the Gene Expression Omnibus (GEO) database of the National Center for Biotechnology Information (NCBI) (accession no. PRJNA213008). RNA-Seq paired-end data were mapped to *A. pisum*’s genome, version 2.0 (aps\_ref\_Acyr \_2.0\_chrUn), with 16, 919 RefSeq genes using HISAT2 v2.1.0 (*6*). Aligned reads were assembled and quantified using StringTie v1.3.4 (*7*) following developer’s protocol. Raw transcript read counts for each sample were normalized and analyzed using DESeq2 v1.19.38 (*8*). Differentially expressed genes between bacteriocytes of both host plant samples were identified using likelihood ratio test based on generalized linear model (~host plant type + tissue type vs. ~host plant type) in DESeq2 v1.18.1 (*8*). Statistical significance was determined if FDR adjusted p-values were ≤ 0.05 and when a greater than 1.5x change in normalized read count values between samples. To identify the genes that are differentially expressed in bacteriocytes of different host plants likelihood ratio test was performed between alfalfa bacteriocytes and fava bacteriocytes (ABAC vs. FBAC) based on read counts using DESeq2 v1.18.1 (*8*). Statistical significance was determined if the adjusted p-value ≤ 0.1 and when a greater than 1.5x change in normalized read count values between samples.

Aphid amino acid pathways and putative enzyme functions were analyzed using the *A. pisum* genome and annotations in the NCBI, Kyoto Encyclopedia of Genes and Genomes (KEGG) (*9*), BRENDA Enzyme Information System (*10*), EcoCyc (*11*), and AcypiCyc (*1*) databases. Gene Set Enrichment Analysis (GSEA) (*12*) was used to determine which KEGG pathways were differentially regulated at the normalized p < 0.1 and p < 0.05, as described in Hansen and Degnan (*13*).

**DNA isolation and whole genome bisulfite sequencing**

For whole genome bisulfite sequencing trials, the same six sub-lines (F1, F2, F3, A1, A2, A3) were prepared as described above for RNA-Seq trials. Dissections were carried out similar to RNA-Seq trials above, except DNA, instead of RNA, was isolated and extracted with the Master Pure Kit (Epicentre Technologies, Madison, WI) following manufacture guidelines. After DNA was extracted, DNA concentrations of each sample were quantified by Qubit dsDNA HS kit (Invitrogen, Carlsbad, CA). 200 ng of DNA was used for bisulfite conversion with the EZ DNA Methylation-LightningTM Kit (Zymo Research, Irvine, CA). Genomic DNA was spiked with 0.5 ng of λ phage DNA (New England BioLabs, Ipswich, MA) as a control since it is unmethylated at CpG sites to calculate the rate of false positives from the sodium bisulfite conversion treatment and to control for the background conversion rates. The bisulfite libraries were constructed using the EpiGnomeTM Methyl-Seq Kit (Epicentre Technologies, Madison, WI) with the starting material of 50 ng of bisulfite-treated DNA following the manufacturer’s guidelines. Strand-specific sequencing was conducted on HiSeq 2500 (Illumina, San Diego, CA) using a TruSeq SBS sequencing kit (Illumina). Reads for all DNA samples (12 total: six bacteriocyte and six corresponding body tissue samples) were submitted to the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) (accession no. PRJNA339317).

**Methylation data analysis**

Raw read data from whole genome bisulfite sequencing were trimmed to remove Illumina index sequences using Trimmomatic (*14*). Methylation read data were aligned to *A. pisum*’s genome, version 2.0 with Bismark as suggested in (*15*). The methylation level at each CpG site with 10 reads or greater was determined by the number of Cs at a given site in the mapped reads divided by the total number of reads. To standardize the sample size of CpG sites per sample, only shared CpG sites that were ≥ 10 reads per CpG site for all 6 biological replicates were evaluated in this analysis. After standardization, percent methylation (methylated versus unmethylated reads) per sample was calculated by averaging every site-specific methylation percentage within a sample. A paired t-test was used to determine if the percent methylation of CpG sites were significantly different between bacteriocyte and body samples (BAC vs. BODY) and between bacteriocytes of alfalfa and fava feeding aphids (ABAC vs. FBAC). To compare how similar CpG methylation profiles are among samples, PCA analysis was conducted with site-specific CpG methylation data for each sample using the methylKit package in R (*16*). To test the within-group dispersion of CpG methylation profiles for each group, multi-response permutation procedure was used as suggested in (*17*) using vegan package in R (*18*). The four groups were defined as: *1)* bacteriocytes of alfalfa-treated aphids (ABAC), *2)* body cells of alfalfa-treated aphids (ABODY), *3)* bacteriocytes of fava-treated aphids (FBAC), and *4)* body cells of fava-treated aphids (FBODY). The average within-group distances were calculated using Sorensen distances.

To determine if the percent methylation of CpG sites was significantly different between the different genic regions (exon, intron, and intergenic regions) average percent methylation was compared between each genic region per sample using a paired t-test. To determine if a methylated CpG site was significantly higher (hyper-methylated) or lower (hypo-methylated) in percent methylation between tissue samples (i.e. differential methylation) a beta binomial model was used to test for significant differences between bacteriocytes and body cells (BAC vs. BODY) and between bacteriocytes of alfalfa and fava feeding aphids (ABAC vs. FBAC) using methylSig package v0.4.4 in R (*19*). Methylation levels at a specific cytosine site were determined to be significantly different between bacteriocyte and body tissues if there was ≥ 10 % difference in percent methylation between groups and the FDR adjusted p-value was q ≤ 0.01.

To link DNA methylation profiles in aphid tissue treatments to differential gene expression patterns, we combined RNA-Seq data collected from the corresponding aphid tissue samples with the methylation data (N=6 biological replicates) (see methods above). Differentially spliced genes were identified as significantly expressed between bacteriocyte and body tissues (BAC vs. BODY) and between bacteriocytes of alfalfa and fava feeding aphids (ABAC vs. FBAC) using the FDR adjusted p-values (q≤ 0.05), based on the normalized read counts generated by HISAT2 v2.1.0 (*6*). Statistical comparison of different exon usages was conducted as described in (*20*) using DEXSeq package v1.24.4 in R (*20)*, with a false discovery rate criterion for calculating p-values. Statistical significance was determined if FDR adjusted p ≤ 0.05. Genes that were both differentially methylated and differentially expressed/spliced were characterized following the same criteria that was used for the RNA-Seq data analysis using GSEA *(12*) (see above). GSEA (*12*) was used to determine which KEGG pathways (*9*) and gene ontology (GO) terms were both differentially regulated and methylated at the normalized P ≤ 0.05. Only groups with two or more genes within a KEGG pathway group were analyzed. Significant GSEA groups (GO terms and KEGG pathways) were further analyzed using the *A. pisum* genome and annotations in the NCBI, KEGG (*9*), BRENDA Enzyme Information System (*10*), and AcypiCyc (*1*) databases.

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