

Supporting Information

Wier et al. 10.1073/pnas.0909712107

SI Methods

RNA Preparation. Messenger RNA was prepared from total RNA extracted from the samples at each of four time points (0400, 1000, 1600, and 2200 hours) during the day. For each of the three replicate samples at each time point, pools of 12 light-organ central cores were dissected from 6 adult squid, and homogenized with a Polytron 1200C (Brinkmann Instruments), for a total of 36 central cores from 18 individual specimens at each of the four times. Pooling of light-organ cores before extraction was necessary to obtain sufficient RNA for robust hybridizations on the arrays. In addition to these three biological replicates of the four time points, dye-swap replicates and two on-chip replicates were performed to control for labeling bias. In all, there were a total of at least four technical replicates for the individual biological replicates at each time-point.

Total RNA was extracted using the MasterPure Purification Kit (Epicentre Biotechnologies), followed by the RNeasy Mini Kit with on-column DNase digestion (Qiagen). An additional off-column DNase (Promega) digestion was also performed. The resulting RNA sample was concentrated with a Microcon-30 spin-filter (Millipore). The concentration and quality of the samples were determined using a NanoDrop spectrophotometer and a Bioanalyzer RNA chip (Agilent Technologies) according to the manufacturer's instructions. Each biological replicate, consisting of 12 light-organ central cores, contained an average of 20 μg of total RNA (comprising both host and symbiont pools). Visual inspection of the Agilent-generated electropherogram profiles for each sample revealed distinct ribosomal bands and no evidence of degradation products. Messenger RNA, used for the host microarrays was purified using a Qiagen Oligotex Mini kit according to the manufacturer's instructions.

Microarray Hybridizations. The spotted microarray used for host analyses contained 13,962 cDNAs applied two times on each glass slide, for a total of 27,924 sample spots (1). The microarray slides had 5 positive-control spikes based on sequences from *Xenopus laevis*, *Anopheles gambiae*, *Schistosoma mansoni* and *Apis mellifera*. These sequences did not cross-hybridize with any of the cDNAs from the *E. scolopes* light organ EST database. Negative controls were spots of (i) buffer alone, (ii) polyA oligonucleotides, or (iii) no template.

Each spotted-array experiment was performed with 50 ng of host mRNA. The mRNA samples were processed and indirectly labeled with 3DNA Array 350 Cy3 and Cy5 kits (Invitrogen). On each slide, we used a run-off reference, a mixture of vector-primed transcription products derived from an equal mixture of the 13,962 *E. scolopes* cDNA clones, labeled with the same protocol as the samples (see below). Each spotted glass-slide microarray was hybridized with two samples: (i) the experimental cDNA; and (ii) the run-off reference, using a procedure described in ref. 2, except that sheared *E. scolopes* genomic DNA was used to block repetitive elements. This genomic DNA was isolated from a freshly dissected adult squid using the Blood and Cell Culture DNA Maxi Kit (Qiagen). As a control for technical variation inherent in the fluorescent-probe chemistry, the Cy3 and Cy5 labeling of the experimental sample and the run-off reference were alternated in at least two slides per replicate (i.e., 3 replicates per time point).

For the symbiont analyses, samples of total light-organ RNA (symbiont + host) from the four time points (three biological replicates each) were used to prepare biotin end-terminus labeled cDNA, which was hybridized to an Affymetrix GeneChip, and scanned on a GC3000 scanner. The protocol and procedures used

are described in the Affymetrix GeneChip Expression manual (www.affymetrix.com). Results from the means of the triplicate samples were analyzed using the Affymetrix Microarray Suite v.5 and Cyber-T (<http://cybert.microarray.ics.uci.edu>). We sorted for genes that showed >1.5-fold differential regulation between at least two consecutive time points, with a $P < 0.01$. Although the statistical significance of the differences was robust, the absolute value of the fold-regulation was relatively low, possibly due to (i) contaminating host mRNA, and (ii) genomic divergence between the dozens of *V. fischeri* strains (3) contributing to the light-organ RNA preparations, and the strain (4) used to design the microarray features.

Analysis of Host Microarray Significance. Expression-level results for the glass-slide microarrays were first normalized to the run-off reference; additional analyses were performed with GeneSpring GX software (Agilent Technologies). To improve confidence in the results, the data were further normalized independently using a per-spot per-chip (PSPC) analysis. To eliminate systematic error resulting from signal-intensity levels below a reliable range, the cutoff was set at 100 units (range = 100–319,623). To control for inconsistency between replicates, both raw and control data were filtered with 50% confidence according to the cross-gene error model (5). The analyses revealed 1,723 transcripts/genes that appeared to be differentially regulated between at least one pair of consecutive sampling times. These candidates were further filtered against two criteria: (i) a fold-change threshold of >2.0 between consecutive time points, and (ii) an analysis of variance (ANOVA) with no multiple-test correction that gave a p value ≤ 0.05 . Both criteria were fulfilled by 1,336 transcripts/genes (Tables S1 and S2) that were considered significantly differentially regulated between at least two of the sampling time points.

Analysis of Symbiont Microarray Significance. A mask was created to exclude the two other genomes present on the Affymetrix GeneChip, and CEL files were imported into GeneSpring GX software (Agilent Technologies). The statistical significance of the data were assessed by first performing a GC-RMA (Robust Multi-Array) normalization on all chips. All data values less than 0.01 were transformed to 0.01, and each chip was normalized to its median intensity. As a further level of stringency, we required that flags either be present or marginal in at least two out of three replicates, resulting in a data set that included 3,472 (>92%) of the *V. fischeri* ORFs. A parametric test was performed, with variance assumed equal (ANOVA) and a p value cut off of 0.05, with no multiple-test correction. This relaxed stringency level allowed us to capture a larger share of the genes representing the different metabolic states that the bacterial population alternates between. The resulting 843 ORFs were filtered on a fold-change of >1.5 between at least one pair of consecutive sampling times, creating a list of 655 ORFs (Tables S1 and S3) that were considered significantly differentially regulated between at least two of the sampling time points.

Quantitative Real-Time PCR. For verification of the quality of the host microarray, we chose to examine five specific genes of interest (e.g., several cytoskeletal genes), as well as five other randomly selected candidates. Each gene was assayed in triplicate by one-step QRT-PCR performed on a LightCycler 480 Thermal Cycler (Roche Applied Science) using the Access RT-PCR system (Promega). Gene-specific primers were designed to create a product between 80 and 150 bp (Table S5), and amplification was

performed under the following conditions: 95 °C for 5 min, followed by 50 cycles of: 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 15 s. Each reaction was performed in triplicate, and contained 0.2 μM primers and 3.0 mM MgCl₂ in the Access RT-PCR System buffer. To determine whether a single amplicon resulted from this PCR, the presence of only one optimal dissociation temperature was achieved by incrementally increasing the temperature every 10 s, from 60 to 89.5 °C. Standard curves were created using a 10-fold cDNA dilution series with each primer set, and the fold-change in transcript abundance was calculated between each time interval (6). The efficiencies of all QRT-PCR reactions were between 90% and 105%; the range between any two reactions used to determine fold-change was <10%, and the triplicate measurements obtained were within 1/2 cycle of each other. All reactions used to determine fold-change were constructed from the same set of cDNA dilutions. Data were interpreted in Qbase (7).

Transmission Electron Microscopy (TEM) of Host Tissue Samples. Adult animals were killed in 4% glutaraldehyde in 0.1% sodium cacodylate with 0.45 M NaCl, pH 7.4, (Fixative A) at four different times during the day/night cycle: 0400, 1000, 1600, and 2200 hours. Light organs were dissected out of the mantle cavity and placed in Fixative A for 12 h, rinsed in 0.45 M NaCl in 0.1 M sodium phosphate at pH 7.4 (Buffer A) for 1 h, and then postfixed in 1% osmium tetroxide in Buffer A for 1 h, followed by rinsing with fresh Buffer A for 30 min. Samples were then dehydrated through a graded ethanol series, and infiltrated with propylene oxide and unaccelerated Spurr (8) for 1 h. They were then transferred first to 100% unaccelerated Spurr for 24 h, and then to 100% accelerated Spurr for 24 h. Samples were embedded in freshly prepared accelerated Spurr at 67 °C for 48 h. For TEM examination, the embedded samples were sectioned, stained with Reynolds lead citrate solution and 3% uranyl acetate, and viewed with a JEOL CX-100 transmission electron microscope. For histological examination, 1-μm thick sections were stained with 1%

toluidine blue and viewed under a Zeiss Axiophot compound light microscope.

Preparation of Symbionts and Host Tissue for Fatty Acid Analysis. Ten adult light-organ central cores were removed at noon as described above, and gently homogenized in 1 mL of 50% Instant Ocean (IO; Spectrum Brands Co.) at 0 °C to release the symbionts (9). The homogenate was cleared of host-tissue debris by two low-speed centrifugations (1,500× g, 15 min). The supernatant fluid, containing most of the symbionts, was then centrifuged at high speed (14,000× g, 1 min) to collect the bacterial cells. The bacterial pellet was extracted once with Triton X-100 (0.5 mg per ml 70% IO) to disperse any lipid-vesicle contamination, and the symbiont cells pelleted and washed again with 70% IO at 0 °C. A small portion of the symbionts was also inoculated into 10 mL of LBS medium (3), and the culture grown for eight generations, after which the bacteria were harvested by centrifugation.

Fatty Acid Analyses. Phospholipids were extracted for fatty-acid compositional analysis as described in Lai and Cronan (10). Briefly, cell pellets were extracted using the method of Bligh and Dyer (11), and the resulting chloroform layer was washed with KCl, and then with water, before solvent evaporation under N₂. The extracted phospholipids were dissolved in dry methanol containing sodium methoxide (10). After incubation and acidification, the fatty-acid methyl esters (FAMES) were extracted with petroleum ether. FAMES were separated and analyzed on an Agilent 6890N gas chromatograph system equipped with an Agilent 5973 mass detector. The separations were performed using a Phenomenex ZB-Waxplus column (30 m in length, 0.25 mm internal diameter and 250 μm film thickness). Each mass spectrum was identified by comparison with FAME standards compiled in a custom library by the Roy J. Carver Metabolomics Center (University of Illinois Urbana-Champaign). The quantitative analysis of each FAME was obtained relative to an internal standard of methyl heptacosanoate (Sigma-Aldrich).

1. Chun CK, et al. (2008) Effects of colonization, luminescence, and autoinducer on host transcription during development of the squid-vibrio association. *Proc Natl Acad Sci USA* 105:11323–11328.
2. Peng X, et al. (2003) Statistical implications of pooling RNA samples for microarray experiments. *BMC Bioinformatics* 4:26.
3. Wollenberg MS, Ruby EG (2009) Population structure of *Vibrio fischeri* within the light organs of *Euprymna scolopes* squid from Two Oahu (Hawaii) populations. *Appl Environ Microbiol* 75:193–202.
4. Ruby EG, et al. (2005) Complete genome sequence of *Vibrio fischeri*: a symbiotic bacterium with pathogenic congeners. *Proc Natl Acad Sci USA* 102:3004–3009.
5. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I (2001) Controlling the false discovery rate in behavior genetics research. *Behav Brain Res* 125:279–284.
6. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29:e45.
7. Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J (2007) qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biol* 8:R19.
8. Spurr AR (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. *J Ultrastruct Res* 26:31–43.
9. Ruby EG, Asato LM (1993) Growth and flagellation of *Vibrio fischeri* during initiation of the sepiolid squid light organ symbiosis. *Arch Microbiol* 159:160–167.
10. Lai CY, Cronan JE (2003) Beta-ketoacyl-acyl carrier protein synthase III (FabH) is essential for bacterial fatty acid synthesis. *J Biol Chem* 278:51494–51503.
11. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37:911–917.

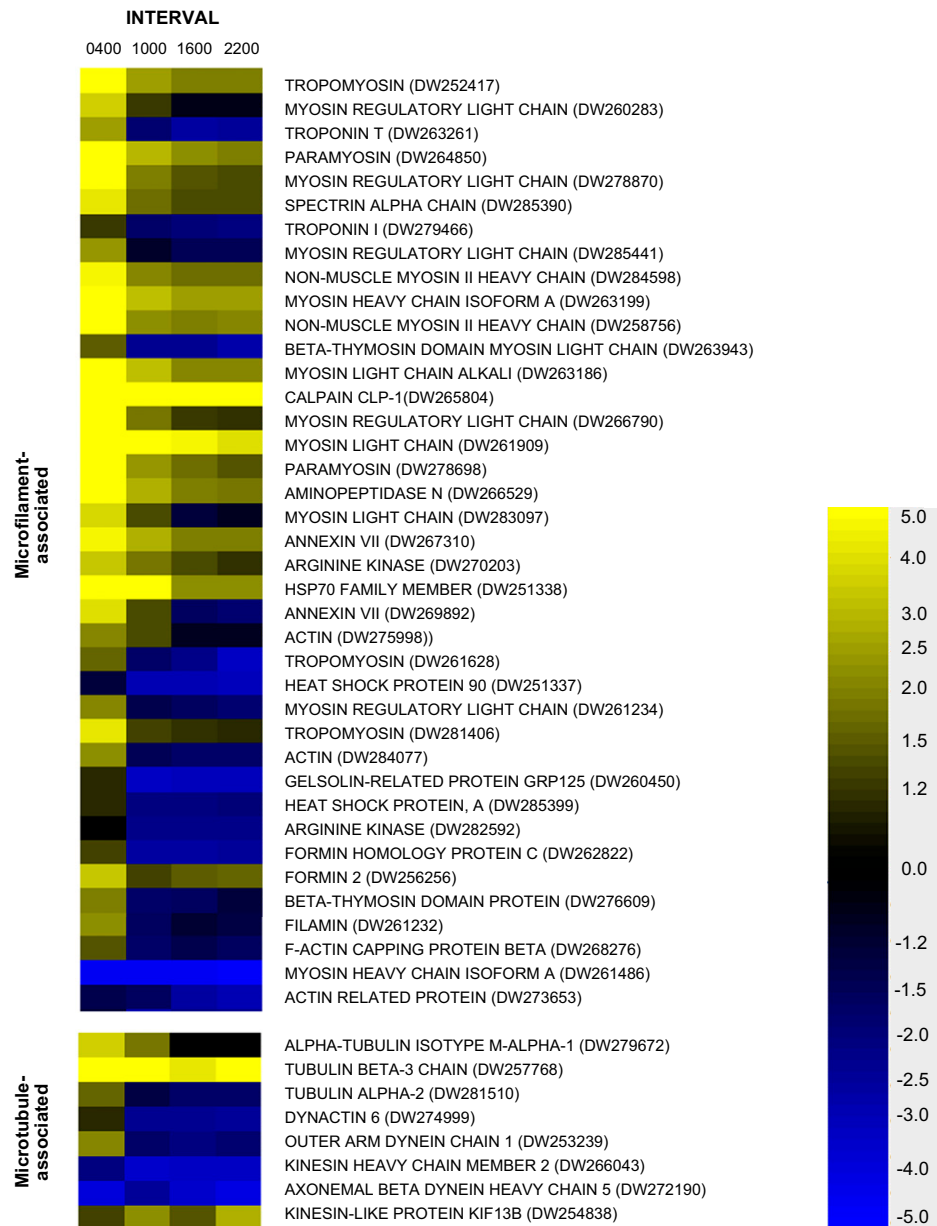


Fig. S1. Heat map of cytoskeletal gene expression in the host. The relative transcript levels (indicated by the color bar) at four times of day (0400, 1000, 1600, and 2200 hours) are indicated for each of the differentially expressed microfilament- and microtubule-associated genes in the *E. sciolepes* cDNA library.

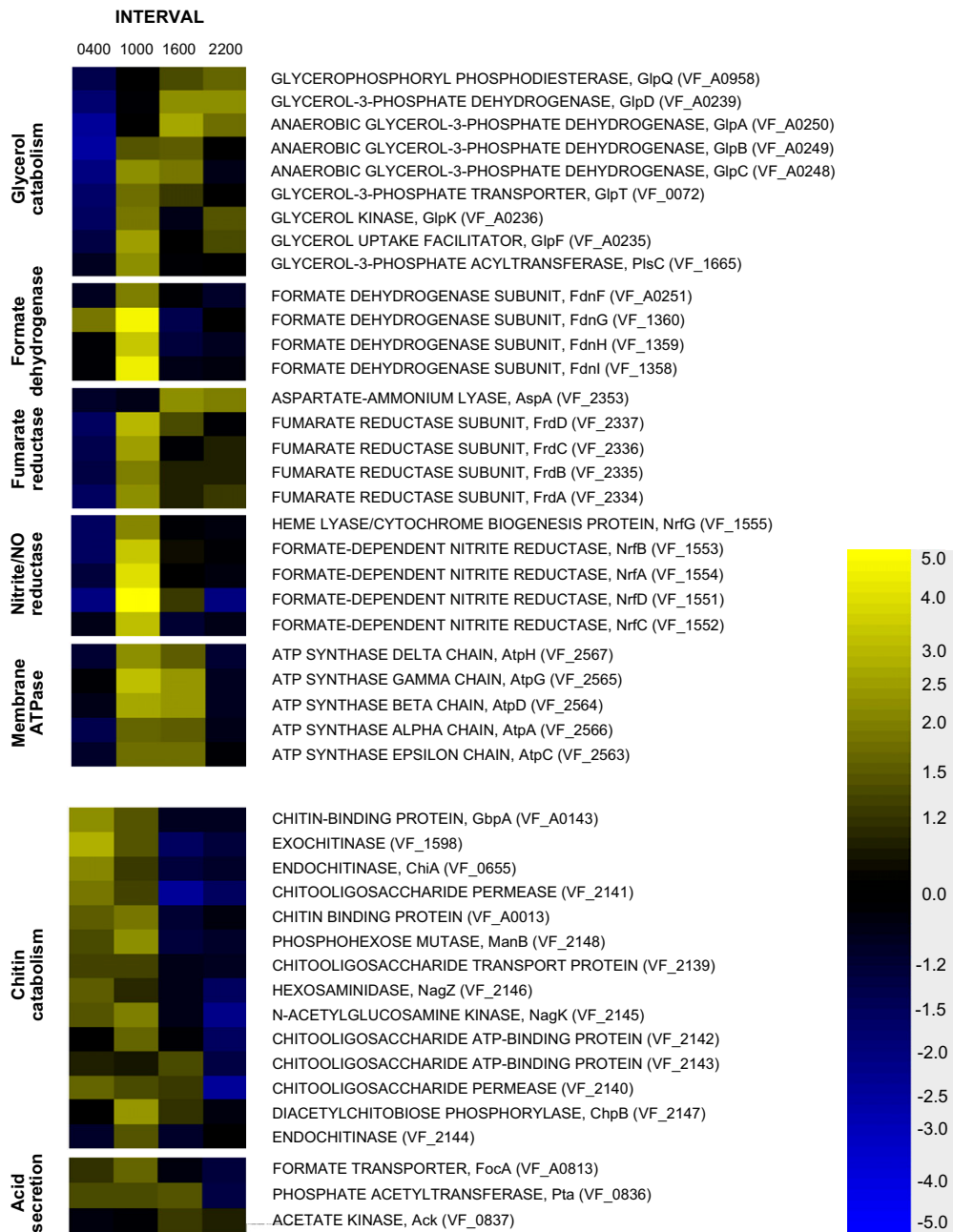


Fig. S2. Heat map of metabolic gene expression in the symbiont. The relative transcript levels (indicated by the color bar) at four times of day (0400, 1000, 1600, and 2200 hours) are indicated for five sets of *V. fischeri* genes active in the anaerobic respiration of glycerol, and two sets of *V. fischeri* genes active in the fermentation of chitin.

Other Supporting Information Files

[Table S1 \(DOC\)](#)

[Table S2 \(XLS\)](#)

[Table S3 \(XLS\)](#)

[Table S4 \(DOC\)](#)

[Table S5 \(DOC\)](#)

[Table S6 \(DOC\)](#)

Table S1. Classes of host and symbiont light-organ transcripts that are differentially regulated over a 24-h period

<i>Euprymna scolopes</i> Number of transcripts differentially regulated ≥ 2 -fold					
Time Interval (h)	Total†	Up	Down	Known‡	Unknown/ Hypo‡
#1 (1600 – 2200)	22	8	14	13	9
#2 (2200 – 0400)	1,066	956	101	535	531
#3 (0400 – 1000)	1,145	172	973	549	596
#4 (1000 – 1600)	112	7	105	52	60

<i>Vibrio fischeri</i> Number of transcripts differentially regulated ≥ 1.5 -fold					
Time Interval	Total†	Up	Down	Known‡	Unknown/ Hypo‡
#1 (1600 – 2200)	112	68	44	86	26
#2 (2200 – 0400)	249	114	135	177	72
#3 (0400 – 1000)	424	254	170	332	92
#4 (1000 – 1600)	351	137	214	271	80

†Those transcripts that are either up- or down-regulated, calculated as the level at the latter time of day divided by that at the former.

‡Known, related to a described gene; unknown/hypo, either undescribed or hypothetical protein-encoding gene with no hits, and no significant homology to the nonredundant database of Genbank, as determined by BLASTX analysis

Table S4. Categories of host and symbiont genes regulated over the day-night cycle*

	Time interval #1 1600-2200 h			Time interval #2 2200-0400 h			Time interval #3 0400-1000 h			Time interval #4 1000-1600 h		
Host gene categories	Up	Down	Total	Up	Down	Total	Up	Down	Total	Up	Down	Total
Metabolism [†]	0	5	5	139	5	144	15	117	132	1	11	12
Cytoskeletal	0	1	1	38	1	39	4	38	42	0	1	1
Immune/Response to Stress	0	0	0	25	3	28	4	20	24	0	5	5
Information Storage/Processing [‡]	3	0	3	91	4	95	12	85	97	1	13	14
Signal Transduction/Regulation [§]	1	1	2	31	0	31	4	30	34	0	7	7
Miscellaneous [¶]	0	2	2	183	15	198	30	190	220	1	12	13
Total known genes	4	9	13	507	28	535	69	480	549	3	49	52
	1600-2200 h			2200-0400 h			0400-1000 h			1000-1600 h		
Symbiont gene categories	Up	Down	Total	Up	Down	Total	Up	Down	Total	Up	Down	Total
Metabolism	21	8	29	28	35	63	77	57	134	27	67	94
Cell Structure	11	4	15	9	25	34	32	25	57	15	23	38
Defense	0	0	0	0	4	4	3	0	3	2	3	5
Information Storage/Processing [‡]	5	4	9	17	10	27	34	28	62	23	29	52
Signal Transduction/Regulation [§]	9	5	14	9	14	23	25	14	39	13	22	35
Total known genes	46	21	67	63	88	151	171	124	295	80	144	224

*Categories for differentially expressed host and symbiont genes with known annotations having a >2.0 and >1.5 fold change, respectively, at consecutive time-point comparisons.

†Includes enzymes and mitochondrial genes.

‡Includes transcription, translation and ribosomal genes.

§ Includes kinase, phosphatase, calcium-related genes, and genes related to protein-turnover.

¶Annotated genes not associated with a designated category.

Table S5. Host microarray verification

Gene	Interval	Fold change*		Primer sequences	Product size†
		Microarray	QRT-PCR		
Annexin DW267310	1600-2200	1.0	1.3	Forward: 5' TCCGCCGCGAATGTTCTGGAATA Reverse: 5' AGTGAATCATTGTGCCAGCTCC	131 bp
	2200-0400	2.4	2.1		
	0400-1000	-1.7	-2.8		
F-Actin DW268276	1000-1600	-1.5	1.1	Forward: 5' TGTGAATGGGCTGAGGTCAGTGAT Reverse: 5' GTGCCATCACCAAGTCATTACGCA	80 bp
	1600-2200	1.1	1.0		
	2200-0400	2.2	1.5		
Actin DW275998	0400-1000	-2.3	-2.0	Forward: 5'TCCATTGTCCACCGCAAATGCTTC Reverse: 5'ACAAGAACTTCCTTCGTCCAGCGT	116 bp
	1000-1600	1.2	1.3		
	1600-2200	1.1	-1.4		
Beta-Catenin DW255108	2200-0400	6.9	7.9	Forward: 5' AGTGACGTTTGATCCGAAGGTGGT Reverse: 5' ATTGAGGTCCGCGATCAACAAACG	148 bp
	0400-1000	-2.9	-2.8		
	1000-1600	-2.2	-2.1		
CrystallinJ1* DW264168	1600-2200	-1.1	-1.4	Forward: 5' TTATGGCGACCAAGCCTATGTGGT Reverse: 5' ATAGGCTGAGGAAGGGCCAAAGAA	112 bp
	2200-0400	2.1	1.1		
	0400-1000	-2.5	-1.2		
78kDA Glucose DW284771	1000-1600	1.1	1.6	Forward: 5' ACGTCTGATTGGAGATGCGGCTAA Reverse: 5' ACTTATCATCCCAGGTGCGACCAA	101 bp
	1600-2200	1.0	1.2		
	2200-0400	1.7	1.9		
HSP-90 DW284163	0400-1000	-1.4	-1.8	Forward: 5' AATGCCTGAAGATGAGGAGGCCAA Reverse: 5' AGCAGGGAGATTCCACAAGACGAT	143 bp
	1000-1600	-1.2	-1.2		
	1600-2200	1.2	-1.2		
LPS-beige DW283155	2200-0400	2.1	2.3	Forward: 5' AAGCCACCCTCACTGGACATCAAA Reverse: 5' TGGAGCCGCTGACAATAATCCAA	81 bp
	0400-1000	-1.5	-1.5		
	1000-1600	-1.2	-1.4		
Rac1* DW283756	1600-2200	1.0	1.0	Forward: 5' AAGCCACCCTCACTGGACATCAAA Reverse: 5' TGGAGCCGCTGACAATAATCCAA	81 bp
	2200-0400	-2.6	-2.3		
	0400-1000	3.5	3.6		
Thioredoxin DW265014	1000-1600	-1.3	-1.5	Forward: 5' TCCTCAGCAGACGATGTTGTCCA Reverse: 5' GGTGGCATGACTTGTGCGGATAAT	81 bp
	1600-2200	-1.1	-1.2		
	2200-0400	2.3	-1.2		
CONTROL Serine HMT DW268753	0400-1000	-1.8	1.3	Forward: 5' TCAACCTCAAGGGAGACGTTATCT Reverse: 5' TCCAATCCTTAAGGGCTGCTGCTT	81 bp
	1000-1600	-1.4	1.0		
	1600-2200	-1.1	-1.3		
Serine HMT DW268753	2200-0400	5.4	5.6	Forward: 5' GTCCTGGTGACAAGAGTGCAATGA Reverse: 5' TTCCAGCAGAAAGGCACGATAGGT	148 bp
	0400-1000	-5.0	-2.4		
	1000-1600	-1.2	-1.8		

* QRT-PCR values are the mean of triplicate measurements, which were all within 1/2 cycle of each other; values are indicated as >2-fold **up-regulated** or **down-regulated**.

† Efficiency was between 90 and 105% at an annealing temperature of 60°C.

Table S6. Differential regulation of symbiont metabolism*ANAEROBIC RESPIRATION OF GLYCEROL/GLYCEROPHOSPHATE

<u>ORF</u>	<u>Interval no.[†]</u>				<u>Annotation</u>
	1 (1600-2200)	2 (2200-0400)	3 (0400-1000)	4 (1000-1600)	
<u>Electron sources</u>					
<i>Glycerol/glycerophosphate catabolism</i>					
VF0072_at*	-1.1	-1.6	2.1	-1.2	Glycerol-3-phosphate transporter (GlpT)
VFA0235_at	1.1	-1.6	2.2	-1.6	Glycerol uptake facilitator protein (GlpF)
VFA0236_at	1.3	-1.9	2.1	-1.5	Glycerol kinase (GlpK)
VFA0239_at	1.0	-2.5	1.6	1.6	Glycerol-3-phosphate dehydrogenase (GlpD)
VFA0248_at	-1.5	-1.8	2.9	-1.1	Anaerobic glycerol-3-phosphate dehydrogenase subunit C (GlpC)
VFA0249_at	-1.2	-2.5	2.9	1.1	Anaerobic glycerol-3-phosphate dehydrogenase subunit B (GlpB)
VFA0250_at	-1.3	-3.1	2.4	1.7	Anaerobic glycerol-3-phosphate dehydrogenase subunit A (GlpA)
VFA0958_at	1.1	-1.8	1.4	1.1	Glycerophosphoryl diester phosphodiesterase (GlpQ)
VF1665_at	1.0	-1.1	1.7	-1.5	1-acyl-sn-glycerol-3-phosphate acyltransferase (PlsC)
<i>Formate dehydrogenase</i>					
VF1358_at	1.0	1.1	2.4	-2.6	Formate dehydrogenase, cytochrome B556 gamma subunit (FdnI)
VF1359_at	1.1	1.1	2.0	-2.5	Formate dehydrogenase N, iron-sulfur beta subunit (FdnH)
VF1360_at	1.4	1.3	1.9	-3.6	Formate dehydrogenase N, cytochrome b-like alpha subunit (FdnG)
VFA0251_at	-1.1	1.0	1.6	-1.4	Formate dehydrogenase H (FdnF)
<u>Proton pumps</u>					
<i>Nitrite/NO reductase</i>					
VF1551_at*	-2.2	1.1	5.0	-2.4	Formate-dependent nitrite reductase (NrfD)
VF1552_at	1.1	1.0	2.0	-2.2	Formate-dependent nitrite reductase, thiosulfate reductase (NrfC)
VF1553_at	-1.1	-1.5	2.9	-1.9	Formate-dependent nitrite reductase, cytochrome c-type protein (NrfB)
VF1554_at	-1.1	-1.2	2.8	-2.2	Formate-dependent nitrite reductase, cytochrome c552 (NrfA)
VF1555_at	-1.0	-1.5	2.2	-1.5	Heme lyase/cytochrome c-type biogenesis protein (NrfG)

Fumerate reductase

VF2334_at	1.0	-1.7	2.3	-1.4	Fumarate reductase flavoprotein subunit (FrdA)
VF2335_at*	1.0	-1.4	1.9	-1.4	Fumarate reductase flavoprotein subunit (FrdB)
VF2336_at	1.1	-1.5	2.3	-1.6	Fumarate reductase flavoprotein subunit (FrdC)
VF2337_at	-1.2	-1.5	2.8	-1.5	Fumarate reductase flavoprotein subunit (FrdD)
VF2353_at	-1.1	-1.6	1.1	1.7	Aspartate-ammonium lyase (AspA)
VF2064_at	-1.1	-1.3	2.1	-1.4	Anaerobic C4-dicarboxylate transporter (DcuB)

Cytochrome oxidase

VF0953_at*	1.1	-1.1	1.3	-1.2	Cytochrome d ubiquinol oxidase subunit I (CydA)
VF0954_at*	1.5	-1.3	1.2	-1.4	Cytochrome d ubiquinol oxidase subunit II (CydB)
VF1299_at*	1.0	-1.1	1.3	-1.2	Cytochrome c oxidase ccb3 subunit I (CcoN)
VF1300_at*	-1.2	1.1	1.1	1.0	Cytochrome c oxidase monoheme subunit ccb3 II (CcoO)
VF1301_at*	-1.3	-1.0	1.4	-1.0	Cytochrome c oxidase component (CcoQ)
VF1302_at*	-1.1	1.1	1.3	-1.3	Cytochrome c oxidase diheme ccb3 subunit III (CcoP)

Proton-gradient harvester

ATPase

VF2563_at	-1.4	-1.1	1.6	1.0	ATP synthase epsilon chain (AtpC)
VF2564_at	-1.8	1.0	1.9	-1.1	ATP synthase beta chain (AtpD)
VF2565_at	-1.7	1.1	2.0	-1.2	ATP synthase gamma chain (AtpG)
VF2566_at	-1.3	-1.2	1.7	-1.0	ATP synthase alpha chain (AtpA)
VF2567_at	-1.5	-1.0	1.9	-1.2	ATP synthase delta chain (AtpH)

FERMENTATION OF CHITIN

Electron sources

Chitin utilization

VF0655_at	1.1	1.7	-1.3	-1.4	Endochitinase (ChiA)
VF1598_at	1.1	2.2	-1.4	-1.9	Exochitinase
VF2139_at*	-1.0	1.3	1.0	-1.2	Chitooligosaccharide transporter binding protein
VF2140_at*	-2.7	3.1	-1.1	-1.1	Chitooligosaccharide transporter permease protein subunit
VF2141_at*	1.5	2.1	-1.2	-2.6	Chitooligosaccharide transporter permease protein subunit
VF2142_at	-1.5	1.6	1.3	-1.3	Chitooligosaccharide transporter ATP-binding protein subunit
VF2143_at*	-1.6	1.4	-1.0	1.1	Chitooligosaccharide transporter ATP-binding protein subunit
VF2144_at*	1.2	-1.1	1.4	-1.4	Endochitinase
VF2145_at	-1.8	2.4	-1.1	-1.5	<i>N</i> -acetylglucosamine kinase (NagK)
VF2146_at*	-1.4	1.9	-1.1	-1.1	Beta-hexosaminidase (NagZ)
VF2147_at	-1.1	1.1	1.5	-1.4	<i>N,N</i> -diacetylchitobiose phosphorylase (ChpB)
VFA0143_at	1.0	1.7	-1.2	-1.4	Chitin-binding protein (GbpA)
VFA0715_at	1.1	1.5	-1.0	-1.5	Chitodextranase precursor

Acid excretion

VF0836_at	-1.6	1.5	1.0	1.0	Phosphate acetyltransferase (Pta)
VF0837_at*	-1.1	-1.1	1.0	1.1	Acetate kinase (Ack)
VFA0813_at*	-1.1	1.4	1.1	-1.4	Formate transporter (FocA)

* ORFs were generally taken from Table S3; those with asterisks were differentially regulated, but didn't meet one of the stringency criteria applied in Table S3: GC-RMA Normalization Genes from all 3 replicates, that were present in at least 9 of 12 samples, with a one-way ANOVA p-value <0.05, and differentially regulated greater or equal to 1.5 fold between at least two consecutive time points.

† up-regulated ≥ 1.2 -fold during the interval; down-regulated ≥ 1.2 -fold during the interval

