Lateral Symbiont Acquisition in a Maternally Transmitted Chemosynthetic Clam Endosymbiosis

Frank J. Stewart,[1] *Curtis R. Young,*[1] *and Colleen M. Cavanaugh*

Department of Organismic and Evolutionary Biology, Harvard University

Deep-sea clams of the family Vesicomyidae live in symbiosis with intracellular chemosynthetic bacteria. These symbionts are transmitted maternally (vertically) between host generations and should therefore show a pattern of genetic variation paralleling that of the cotransmitted host mitochondrion. However, instances of lateral (nonvertical) symbiont acquisition could still occur, thereby decoupling symbiont and mitochondrial phylogenies. Here, we provide the first evidence against strict maternal cotransmission of symbiont and mitochondrial genomes in vesicomyids. Analysis of *Vesicomya* sp. mt-II clams from hydrothermal vents on the Juan de Fuca Ridge (northeastern Pacific) revealed a symbiont phylotype (designated $symB_{VII}$) highly divergent from previously described symbionts of the same host lineage. $SymB_{VII}$-hosting clams occurred at low frequency (0.02) relative to individuals hosting the dominant symbiont phylotype. Phylogenetic analysis of 16S rRNA genes from a wide range of symbionts and free-living bacteria clustered $symB_{VII}$ within the monophyletic clade of vesicomyid symbionts. Further analysis of 3 symbiont loci (*23S*, *dnaK*, and *soxA*) across 11 vesicomyid taxa unambiguously placed $symB_{VII}$ as sister to the symbiont of a distantly related host lineage, *Vesicomya* sp. from the Mid-Atlantic Ridge (98.9% median nucleotide identity across protein-coding loci). Using likelihood and Bayesian model discrimination methods, we rejected the strict maternal cotransmission hypothesis by showing a significant decoupling of symbiont and host mitochondrial (*COI* and *mt16S* genes) phylogenies. Indeed, decoupling occurred even when $symB_{VII}$ was excluded from phylogenetic reconstructions, suggesting a history of host switching in this group. Together, the data indicate a history of lateral symbiont transfer in vesicomyids, with $symB_{VII}$ being the most conspicuous example. Interpreted alongside previous studies of the vesicomyid symbiosis, these results suggest a mixed mode of symbiont transmission characterized by predominantly vertical transmission punctuated with instances of lateral symbiont acquisition. Lateral acquisition may facilitate the exchange of genetic material (recombination) among divergent symbiont lineages, rendering the evolutionary history of vesicomyid symbiont genomes much more complex than previously thought.

Introduction

Symbioses between chemosynthetic bacteria and marine invertebrates represent extreme examples of prokaryote–eukaryote coevolution. These mutualisms dominate the fauna at deep-sea hydrothermal vents and cold seeps, where the invertebrate host facilitates access to substrates (sulfur or methane, oxygen, carbon dioxide) needed for the chemoautotrophic metabolism of the symbiont. In exchange, symbiont carbon fixation supports most, if not all, of the host's nutrition (Stewart et al. 2005). This tight metabolic coupling exerts on each partner a strong selective pressure for maintenance of the interaction. Such coevolutionary interactions might be expected to drive the parallel diversification of chemosynthetic symbionts and hosts (cospeciation), as has been shown in other prokaryote–eukaryote symbioses (Chen et al. 1999; Clark et al. 2000; Lo et al. 2003; Degnan et al. 2004; Wade 2007). However, many marine chemosynthetic symbioses do not show strong patterns of cospeciation (Krueger and Cavanaugh 1997; Di Meo et al. 2000; Nelson and Fisher 2000; Won et al. 2003; Suzuki et al. 2006; Vrijenhoek, Duhaime, and Jones 2007). Notably, symbioses between gamma proteobacteria and heterodont clams of the Vesicomyidae are the only members of this group that show a significant coupling of symbiont and host genetic variation (Peek, Feldman, et al. 1998). These symbioses are therefore useful models for studying bacteria–eukaryote coevolution. This study examines an interesting exception to the general cospeciation pattern in vesicomyids and discusses its implications for inferences of symbiont transmission mode in these taxa.

The Vesicomyidae comprises at least 50 species occurring ubiquitously at vents and seeps throughout the world's oceans (Goffredi et al. 2003; Kojima et al. 2004; Krylova and Sahling 2006). Fossil evidence suggests that this diverse group radiated between 50 and 100 MYA, and all extant species are thought to host sulfur-oxidizing bacterial endosymbionts (Kiel and Little 2006). Several lines of evidence show that both the clam and the symbiont have undergone extensive coadaptation to accommodate life in symbiosis. The host retains only a rudimentary gut, relying instead on symbiont autotrophy for nutrition (Kennish and Lutz 1992). The bacterial symbionts reside within specialized cells of the gills (Cavanaugh 1983; Fiala-Médioni and Métivier 1986) and appear to be obligately symbiotic, as they have never been cultured or detected in the environment. Further, genomic studies show that the single chromosome of vesicomyid symbionts is only approximately 1.1 Mb in size, suggesting that, like the bacterial symbionts of insects (e.g., *Buchnera*; van Ham et al. 2003), vesicomyid symbionts may be experiencing genome reduction as an adaptation to an obligate intracellular lifestyle (Kuwahara et al. 2007; Newton et al. 2007).

The coadapted symbiont–host interaction is presumably maintained across generations by maternal (vertical) transmission of the symbiont via the clam's eggs. Maternal transmission in vesicomyids was first suggested by electron micrographs showing bacteria-like structures in ovarian tissue (Endow and Ohta 1990) and then by molecular probing that localized symbionts to the follicle cells of the primary oocyte (Cary and Giovannoni 1993). Subsequently, maternal transmission has also been inferred using phylogenetic and population genetic studies. Under a strict maternal

[1] F.J.S. and C.R.Y. contributed equally to this work.

Key words: chemosynthetic symbiont transmission, coevolution, cospeciation, *Calyptogena*, cold seep, hydrothermal vent.

E-mail: cavanaug@fas.harvard.edu.

Mol. Biol. Evol. 25(4):673–687. 2008
doi:10.1093/molbev/msn010
Advance Access publication January 12, 2008

transmission hypothesis, the symbiont and host mitochondrial genomes necessarily pass through the same individuals and are completely genetically linked (Hurtado et al. 2003). Patterns in symbiont variation should therefore parallel those of the mitochondrion at both the species and the population level (Clark et al. 2000; Hurtado et al. 2003). Indeed, using maximum likelihood (ML)–based hypothesis testing, Peek et al. (1998) showed a general congruence between symbiont 16S rRNA and host mitochondrial gene phylogenies for 9 vesicomyid taxa, though minor discrepancies occurred at 2 of the 7 internal nodes. Similarly, for clams of the *Vesicomya pacifica/lepta* species complex (eastern Pacific), Goffredi et al. (2003) showed that symbiont and host genealogies exhibited parallel partitions, suggesting a tight coupling of symbiont and host mitochondrial genomes. Further, focusing on intraspecies and intrapopulation variation, Hurtado et al. (2003) demonstrated symbiont–mitochondrion coupling in a population of the vesicomyid *Calyptogena magnifica* that was polymorphic for both symbiont and mitochondrial markers. Together, these studies suggest a hypothesis of nearly complete maternal symbiont transmission in these taxa (Hurtado et al. 2003).

Events that disrupt strict maternal transmission and decouple symbiont and clam phylogenies might still be possible. These include the acquisition of new symbionts from the environment or the horizontal transfer of symbiont lineages between distinct clam hosts (host switching), perhaps via hybridization events. Here, we use the term "lateral" to specify any nonmaternal (i.e., nonvertical) transfer events, including horizontal transfer between hosts and environmental acquisition. A decoupling of symbiont and host phylogenies has been used as evidence of lateral transfer in other symbiotic associations, notably those between insects and maternally transmitted parasitic bacteria of the genus *Wolbachia* (Werren et al. 1995; Schilthuizen and Stouthamer 1997; Haine et al. 2005). For vesicomyids, detection of lateral transfer events would suggest that the specificity of the symbiont–host interaction is not absolute, raising the possibility that a single host lineage could accommodate functionally divergent symbiont lineages (or vice versa) and that vesicomyid clams might be at different stages in the process of completely internalizing their nutritional symbionts. Further, lateral symbiont transfer in these taxa might create opportunities for the exchange of genomic material (recombination) between diverse symbiont lineages (e.g., see Papke and Ward 2004). To date, however, lateral symbiont transfer in vesicomyids has not been shown.

This study shows a significant decoupling of vesicomyid symbiont and host mitochondrial phylogenies, providing the first evidence of lateral symbiont transfer events in the evolutionary history of these clams. Genetic analysis of hydrothermal vent clams from the northeastern Pacific revealed a symbiont phylotype highly divergent from other symbionts of the same host lineage. Multilocus analysis showed that the divergent symbiont clusters tightly with the symbiont from a distantly related host clam. Further, Bayesian analysis using a computationally robust model choice statistic, the Akaike information criterion Monte Carlo (AICM; Raftery et al. 2007), demonstrated incongruence between symbiont and host mitochondrial gene trees even in the absence of the divergent symbiont lineage, suggesting a history of lateral symbiont acquisition in this clam family.

Methods and Materials

Vesicomyid Clam Specimens

This study analyzed clams of the *Vesicomya* sp. mt-II lineage, as named by Goffredi et al. (2003) according to mitochondrial haplotype. *Vesicomya* sp. mt-II clams ($n = 3$) were collected from a hydrothermal vent site on the North Endeavor segment of the Juan de Fuca (JdF) Ridge in 1991. Following the discovery of a unique symbiont lineage in 1 of these clams, we screened an additional 115 clams collected from 6 JdF sites in 1995 and 1999 (table 1). We also obtained samples of gill tissue from 9 other vesicomyid species (table 1), either via direct collection or from collaborators. These samples represent species from vent or seep sites in the Pacific Ocean, the Gulf of Mexico, and the Atlantic Ocean. Clams were typically dissected immediately following collection or frozen whole until dissection and analysis at the home institution.

DNA Extraction, Polymerase Chain Reaction, and Sequencing

Total DNA was extracted from the symbiont-containing gill tissue of each clam using the DNeasy tissue kit (Qiagen, Valencia, CA). Gill DNA extracts were used to polymerase chain reaction (PCR) amplify and sequence portions of 3 host and 4 symbiont loci using the primer sets listed in supplementary table 1 (Supplementary Material online). Host markers included portions of the mitochondrial cytochrome oxidase *c* subunit I gene (*COI*; 729 bp), mitochondrial 16S rRNA gene (*mt16S*; 441 bp), and first internal transcribed spacer (ITS) of the nuclear rRNA operon (*nucITS*; 1,190 bp). These host loci have been used previously to characterize vesicomyids collected from the JdF (Peek et al. 1998; Goffredi et al. 2003). Symbiont markers included portions of the 16S rRNA gene (*16S*; 1,303 bp), the 23S rRNA gene (*23S*; 1,725 bp), and 2 single-copy protein-coding genes, *dnaK* and *soxA* (735 and 594 bp). The *soxA* gene encodes an enzyme in the sulfur oxidation pathway of thiotrophic prokaryotes and is presumably critical to energy generation by vesicomyid symbionts (Friedrich et al. 2001; Newton et al. 2007). The *dnaK* gene encodes a molecular chaperone that aids the refolding of damaged proteins. This housekeeping gene is highly conserved and has been used extensively in bacterial phylogenetics (e.g., Gupta 1998). PCR primers for *dnaK* and *soxA* were developed based on conserved regions in the genomes of 2 vesicomyid symbionts: Candidatus *Ruthia magnifica* and Candidatus *Vesicomyosocius okutanii* (the symbionts of the clams *Calyptogena magnifica* and *C. okutanii*, respectively; Kuwahara et al. 2007; Newton et al. 2007). Following visualization via agarose gel electrophoresis, PCR products were purified using the QIAquick PCR purification kit (Qiagen). Purified products were directly sequenced in both the forward and the reverse directions using standard BigDye techniques. Sequences were

Table 1
Identifications and Collection Sites of Vesicomyid Clams Used in This Study

Host Species	*n*	Year	Location	Habitat	Latitude, Longitude	Dive[a]	Depth (m)
Vesicomya sp. mt-II[b]	3[c]	1991	JdF Ridge, North Endeavor,	Vent	47-57.4N, 129-05.9W	A 2413	2,200
	32	1995	JdF	Vent	47-58.1N, 129-05.2W	ATV	2,200
	4	1999	JdF	Vent	47-57.78N, 129-05.51W	A 3454	2,182
	6	1999	JdF	Vent	47-57.66N, 129-05.62W	A 3456	2,184
	7	1999	JdF	Vent	47-58.11N, 129-05.24W	A 3457	2,175
	34[c]	1999	JdF	Vent	47-57.79N, 129-05.48W	A 3459	2,190
	32	1999	JdF	Vent	47-57.73N, 129-05.55W	A 3462	2,190
Vesicomya sp. MAR	1	2001	Logatchev, Mid-Atlantic Ridge	Vent	14-45.32N, 44-58.79W	A 3668	3,028
Calyptogena magnifica	1	2003	East Pacific Rise	Vent	09-50.88N, 104-17.61W	A 3951	2,507
Calyptogena kilmeri	1	2004	Monterey Canyon	Cold seep	36-46.53N, 122-5.21W	V	970
Calyptogena okutanii[d]	NA		Sagami Bay, Japan	Cold seep	34-57N, 139-12E	HP 305	1,157
Calyptogena ponderosa	1	1992	Gulf of Mexico	Cold seep	27-40.88N, 91-32.10W	J 3276	720
Ectenagena extenta	1	2004	Monterery Canyon	Cold seep	36-40.92N, 122-7.21W	V	1,464
Vesicomya gigas	1	2001	Mendocino Fracture Zone	Cold seep	49-20.06N, 127-39.69W	T 473	1,694
Vesicomya sp. mt-I[b]	1	1999	Monterey Bay	Cold seep	36-47N, 122-5W	V	500–1,000
Vesicomya sp. mt-III[b]	1	2000	Monterey Bay	Cold seep	36-37.79N, 122-19.92W	T 217	2,200

[a] Dive: A = *DSV* Alvin, ATV = *ROV* Advanced Tethered Vehicle (Dive 95-48-149, High Rise Expedition), HP = *ROV* Hyper Dolphin, J = *DSV* Johnson Sea Link, T = *ROV* Tiburon, V = *ROV* Ventana (no associated dive numbers).

[b] *Vesicomya* sp. mt-I, -II, and -III are unnamed clam species that have been designated according to mitochondrial type by Goffredi et al. (2003).

[c] Populations in which the divergent symbiont phylotype ($symB_{VII}$) was discovered.

[d] *C. okutanii* host and symbiont sequences obtained from GenBank; collection details correspond to those associated with the sequencing of the *V. okutanii* symbiont genome (Kuwahara et al. 2007).

assembled and edited manually in Sequencher v4.7 prior to phylogenetic analyses. GenBank accession numbers for all sequences obtained in this study are listed in supplementary table 2 (Supplementary Material online).

Phylogenetic Analyses

Multiple gene trees were constructed to describe the symbiont phylotypes in this study. Here, the symbiont phylotype previously characterized for *Vesicomya* sp. mt-II clams is designated $symA_{VII}$, whereas the divergent symbiont phylotype described in this study is designated $symB_{VII}$. The corresponding host haplotypes are $clamA_{VII}$ and $clamB_{VII}$, respectively. The broad phylogenetic placement of $symA_{VII}$ and $symB_{VII}$ was first determined in an analysis of 16S rRNA gene sequences from diverse vesicomyid symbionts ($n = 20$), symbionts of other marine invertebrates ($n = 17$), and several of the most closely related free-living bacteria that appeared in the BlastN results list for the $symB_{VII}$ *16S* sequence ($n = 11$).

To obtain greater phylogenetic resolution, we then conducted a multilocus analysis of 4 symbiont genes (*16S*, *23S*, *dnaK*, and *soxA*) from 9 additional vesicomyid taxa (table 1). The corresponding host mitochondrial gene tree for this taxon set was obtained in an analysis of concatenated *COI* and *mt16S* sequences, as in Peek et al. (1998). *Calyptogena okutanii* was excluded from the mitochondrial analysis, as we did not have *mt16S* data for this species. Additionally, to confirm the identity of $clamA_{VII}$ and $clamB_{VII}$ host clams, *nucITS* sequences from these taxa were analyzed relative to published sequences from clams belonging to the *Vesicomya* sp. mt-I, -II, and -III lineages (see Goffredi et al. 2003). Due to their hypervariability, *nucITS* sequences from the other, more distantly related taxa analyzed in this study could not be unambiguously aligned and were excluded from the analysis. The above analyses were rooted using BlastN to identify the free-living bacterium most closely related to the symbiont taxa. Sequences were aligned in ClustalW (Thompson et al. 1994) and then edited manually in MacClade 4.0 (Maddison DR and Maddison WP 2000).

We first conducted rooted phylogenetic analyses on each locus using ML, maximum parsimony (MP), and Bayesian methodologies. We report only Bayesian phylogenies, as ML and MP phylogenies were not significantly different from those reported (supplementary figs. 2 and 3, Supplementary Material online). A second set of unrooted analyses using the taxa specified in table 1 (excluding *C. okutanii*) was then implemented to test for topological coupling between symbiont and host mitochondrial gene trees (these analyses are described below in Tests for Symbiont–Host Coupling).

Bayesian phylogenetic analyses were conducted on each gene separately using MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). Prior to the analyses, we tested a set of nested models of sequence evolution that are restrictions of the general time reversible (GTR) model with rate variation among sites (Tavaré 1986; Yang 1994). The best-fit model of sequence evolution for each locus was evaluated based on the Akaike information criterion using standard procedures in PAUP v4.0b10 (Akaike 1974; Swofford 1998; Posada and Crandall 2001) and then chosen according to Akaike weights (supplementary tables 3 and 4, Supplementary Material online). Diffuse priors were assumed for all analyses. Under models with site-specific rates, rate parameters (Dirichlet(1,1,1) prior) were assigned to partitions of first, second, and third codon positions. Topology (diffuse prior, all topologies equally weighted), branch lengths (Exponential(10) prior), instantaneous rate matrices (Dirichlet (1,1,1,1,1,1) prior), and equilibrium base frequencies (Dirichlet(1,1,1,1) prior) were shared among the data partitions. Other loci required gamma-distributed rates (α; Uniform(0.05,50) prior) or a proportion of invariant sites

Table 2
Topology Tests Based on Vesicomyid Host[a] and Symbiont DNA Sequence Data

		AICM[b]							BF[c]				
Comparison	Model[d]	Thin	ρ_1	$\hat{\ell}_{max}$	$\hat{d}$	AICM	MCSE	w_i	Log[$f(X\|M_i)$]	Log(BF)	Odds Ratio	P	2log(BF)
w/ sym/clamB_{VII}													
	T_2	2	0.01	−3,380	66	6,825	3.6	0.99	−3,393				
COI versus *mt16S*	T_1	2	−0.03	−3,397	40	6,834	2.2	0.01	−3,408	15	3.3×10^{06}	0.00	30
	T_2	2	0.03	−5,837	76	11,750	4.1	1.00	−5,853				
16S versus host	T_1	2	−0.02	−5,909	50	11,868	2.7	0.00	−5,919	66	4.6×10^{28}	0.00	132
	T_2	2	0.02	−6,658	70	13,385	3.8	1.00	−6,679				
23S versus host	T_1	2	−0.003	−6,761	51	13,572	2.8	0.00	−6,775	96	4.9×10^{41}	0.00	192
	T_2	2	0.04	−5,429	64	10,922	3.5	1.00	−5,444				
dnaK versus host	T_1	2	0.06	−5,659	46	11,363	2.5	0.00	−5,668	224	1.9×10^{97}	0.00	448
	T_2	2	0.006	−5,362	69	10,792	3.7	1.00	−5,376				
soxA versus host	T_1	2	−0.003	−5,547	49	11,143	2.7	0.00	−5,557	181	4.0×10^{78}	0.00	362
w/o sym/clamB_{VII}													
	T_2	2	0.03	−3,361	57	6,779	3.8	1.00	−3,374				
COI versus *mt16S*	T_1	2	−0.01	−3,380	36	6,796	2.5	0.00	−3,391	17	2.4×10^{07}	0.00	34
	T_2	2	−0.01	−5,815	66	11,696	3.6	1.00	−5,832				
16S versus host	T_1	2	0.01	−5,842	46	11,730	2.5	0.00	−5,853	21	1.3×10^{09}	0.00	42
	T_2	2	−0.01	−6,607	65	13,279	3.5	1.00	−6,620				
23S versus host	T_1	2	0.004	−6,630	46	13,305	2.5	0.00	−6,638	18	6.6×10^{07}	0.00	36
	T_2	2	0.03	−5,361	64	10,786	3.5	1.00	−5,377				
dnaK versus host	T_1	2	−0.01	−5,407	47	10,861	2.6	0.00	−5,420	43	4.7×10^{18}	0.00	86
	T_2	2	0.01	−5,320	62	10,702	3.4	1.00	−5,332				
soxA versus host	T_1	2	0.02	−5,354	45	10,753	2.5	0.00	−5,367	35	1.6×10^{15}	0.00	70
	T_2	4	−0.003	−12,979	96	26,053	4.6	1.00	−12,997				
All sym versus host[e]	T_1	4	0.03	−13,034	74	26,143	3.6	0.00	−13,058	61	3.1×10^{26}	0.00	122

[a] Host loci: *COI* and *mt16S* concatenated (1170 bp).

[b] AICM: thin = thinning interval; ρ_1 = first-order autocorrelation of likelihood samples after thinning; $\hat{\ell}_{max}$ = estimate of the maximum likelihood of the data derived from posterior simulation; $\hat{d}$ = estimate of the effective number of parameters; AICM = $2\hat{\ell}_{max} - 2\hat{d}$; w_i = Akaike weights; see Tests for Symbiont–Host Coupling section of Methods and Materials for full details.

[c] BF: log[$f(X|M_i)$] = marginal log-likelihood of the model; log(BF) = difference in support of the models in log-likelihood units; odds ratio = relative probability of the 2 models; P = probability of model; 2log(BF) = values >10 are considered very strong evidence against model T_1 (Kass and Raftery 1995).

[d] Model: T_1 = coupling model that constrains both data sets in the comparison to a single topology; T_2 = decoupling model that allows 2 separate topologies for the data sets being compared.

[e] Symbiont loci (*16S*, *23S*, *dnaK*, *soxA*) concatenated versus host loci (*COI*, *mt16S*).

(I; Uniform(0,1) prior; supplementary table 4, Supplementary Material online).

All Markov chain Monte Carlo (MCMC) analyses were conducted using Metropolis coupling with 20 or more parallel chains. Swap rates between adjacent chains were >20% in all cases. Preliminary MCMC runs were conducted for each analysis to determine appropriate MCMC algorithm tuning, and short runs using optimal tuning parameters were repeated a minimum of 5 times. Two long chains were iterated 5.0×10^6 times, and parameters were sampled every 1,000 iterations. Of these 5,000 samples, the first 1,000 were discarded as burn-in. All repetitions of the analyses converged on very similar parameter estimates, and the 2 long chains were combined for a total of 8,000 posterior samples. MCMC convergence was assessed using the CODA package in R (R Development Core Team 2007). Furthermore, as expected for runs that have converged, the medians of the posterior distributions of model parameters for each locus were all close to ML estimates computed using PAUP (supplementary table 4, Supplementary Material online).

Tests for Symbiont–Host Coupling

Under the prevailing hypothesis of symbiont transmission for vesicomyids, symbionts and host mitochondria are cotransmitted via the egg and should therefore exhibit parallel patterns of genetic divergence (topologies). Coupling between symbiont and mitochondrial topologies was assessed using Bayesian and likelihood methods applied to unrooted data sets containing the vesicomyid taxa in table 1 (excluding *C. okutanii* and its symbiont).

Bayesian analyses compared a coupling model, T_1, in which both host and symbiont loci share the same topology, with a decoupling model, T_2, in which host and symbiont loci were allowed 2 separate topologies (table 2). The analyses were run separately for each symbiont locus concatenated to the 2 host loci (e.g., *dnaK–COI–mt16S*), as well as for a data set containing all 4 symbiont loci concatenated to the 2 host loci (*16S–23S–dnaK–soxA–COI–mt16S*). Single symbiont locus analyses were run both for data sets that contained the divergent symbiont symB_{VII} and its corresponding host clamB_{VII}, as well as for data sets that lacked these sequences. The 6-locus analysis was run only for a data set lacking symB_{VII} and clamB_{VII}. If the data lent greater statistical support to T_2, then a decoupling of symbiont and host mitochondrial phylogenies was indicated.

We used MrBayes to compare the marginal likelihood of the data under models T_1 and T_2. Selection of best-fit nucleotide substitution models for each data partition (locus) was as described above and also involved a second level of assessment to avoid overparameterization and to aid convergence during MCMC runs (see supplementary table 3, Supplementary Material online). Under both T_1

and T_2, each data partition was assigned its own variable rate parameter (Dirichlet(1,1,1,1) prior). MCMC runs were conducted using Metropolis coupling as in the rooted single-gene phylogenetic analyses (above), with the following exception. For the 6-locus analysis, 5 chains (rather than 2) were iterated 5.0×10^6 times applying the same thinning and burn-in as above and then combined for a total of 20,000 posterior samples.

Statistical support for models T_1 and T_2 was assessed using Bayes factors (BF) computed from the harmonic mean of the likelihood scores over the MCMC run (Kass and Raftery 1995). However, the harmonic mean estimator is known to be computationally unstable (Newton and Raftery 1994; Nylander et al. 2004; Raftery et al. 2007), and some researchers have cautioned against its use in phylogenetic analyses (Lartillot and Philippe 2006). Due to this instability, we also applied an alternative model discrimination measure, the AICM (Raftery et al. 2007), using the program MrAICM (Young CR, unpublished data).

AICM is computed from the marginal posterior distribution of log-likelihood scores for the data and is defined as

$$\mathrm{AICM} = 2\hat{\ell}_{\max} - 2\hat{d},$$

where $\hat{\ell}_{\max}$ is an estimate of the ML of the data derived from posterior simulation and $\hat{d}$ is an estimate of the effective number of parameters (i.e., model complexity), also derived from posterior simulation. Posterior simulation–based estimates of these quantities are $\hat{\ell}_{\max} = \bar{\ell} + s_\ell^2$ and $\hat{d} = 2s_\ell^2$, where $\bar{\ell}$ and s_ℓ^2 are the sample mean and variance of the log-likelihood scores, respectively (Raftery et al. 2007). Therefore, AICM is

$$\mathrm{AICM} = 2(\bar{\ell} - s_\ell^2).$$

Raftery et al. (2007) point out that AICM is equivalent to the deviance information criterion as defined in Gelman et al. (2004). The Monte Carlo standard error (MCSE), given B independent MCMC draws, is

$$\mathrm{MCSE}_{\mathrm{AICM}} = \sqrt{\frac{2\hat{d}}{B} + \frac{4\hat{d}}{B}\left(\frac{11\hat{d}}{4} + 12\right)}$$

(Raftery et al. 2007). Subsampling the MCMC data sets every 2–4 simulation replications, depending on the model and the data set, reduced autocorrelation enough so that our samples from the marginal posterior distribution of log-likelihood scores were independent.

We measure the relative support of competing models by computing the Akaike weights, w_i, for each model i (Burnham and Anderson 2002). The best model in a set R of competing models is the one with the maximum AICM, $\mathrm{AICM}_{\max}$. The difference between the best model and other models that we wish to compare is $\Delta\mathrm{AICM}_i = \mathrm{AICM}_{\max} - \mathrm{AICM}_i$, so that the Akaike weights for model i are defined as

$$w_i = \frac{\exp(-(1/2)\Delta AICM_i)}{\sum_{j=1}^{R}\exp\left(-(1/2)\Delta AICM_j\right)}.$$

Symbiont–host congruence was further assessed using methods implemented in the program CONSEL (Shimodaira and Hasegawa 2001). Briefly, the unrooted ML tree for a given symbiont gene was generated via a heuristic search in PAUP using 100 random addition replicates, tree bisection-reconnection branch swapping, and best-fit substitution models chosen as described above (see supplementary table 3, Supplementary Material online, for best-fit models). The sitewise log-likelihoods for this tree were compared with those generated when the same sequence data were constrained to the ML topology of the host data (*COI* + *mt16S*). For the concatenated host data, ML analyses were run under a GTR model with variable rates assigned to each codon position of the *COI* gene and to the *mt16S* gene. Using sitewise log-likelihoods for both constrained and unconstrained topologies, CONSEL was implemented to calculate *P* values according to the approximately unbiased (AU) test using multiscale bootstrapping (Shimodaira 2002), as well as posterior probabilities (PPs) under the Bayesian information criterion (BIC) approximation (Schwarz 1978). *P* values correspond to the probability of obtaining a more extreme test statistic (the difference in log-likelihoods between constrained and unconstrained topologies) by chance under the null hypothesis of no difference between topologies. These analyses were also run reciprocally, that is, host data constrained to the ML topology for each symbiont gene.

Results

Sequence analysis of *Vesicomya* sp. mt-II clams from the JdF Ridge revealed a symbiont phylotype ($\mathrm{symB}_{\mathrm{VII}}$) that was highly divergent from that previously described for this host lineage ($\mathrm{symA}_{\mathrm{VII}}$). This discovery prompted the hypothesis that $\mathrm{symB}_{\mathrm{VII}}$ represented a decoupling of symbiont–host genetic variation and was acquired laterally (nonvertically) by the *Vesicomya* sp. mt-II host lineage. To test these hypotheses, we conducted a multilocus analysis that characterized the phylogenetic placement of $\mathrm{symB}_{\mathrm{VII}}$ and applied statistical tests that confirm symbiont–host decoupling.

Host Clam Phylogeny

Sequencing of 3 host loci (*COI*, *mt16S*, and *nucITS*) unambiguously identified the 3 host clams collected in 1991 from the JdF as members of the *Vesicomya* sp. mt-II lineage of the *V. pacifica/lepta* complex (see naming in Goffredi et al. 2003). Two individuals are identical across both mitochondrial loci and differ from the third individual at only 3 bp of the *COI* sequence (0.41%; supplementary table 5, Supplementary Material online). The third individual, which harbors the divergent symbiont lineage $\mathrm{symB}_{\mathrm{VII}}$ (see below), was identical across both mitochondrial loci to a clam species previously identified as *Calyptogena pacifica* (Vrijenhoek et al. 1994) and later reclassified as *Vesicomya* sp. mt-II (see GenBank AF008295 and AF035732 in Peek et al. (1998) and AY143320 in Goffredi et al. (2003)). We refer to the host haplotype of the first 2 individuals as $\mathrm{clamA}_{\mathrm{VII}}$ and that of the third individual as $\mathrm{clamB}_{\mathrm{VII}}$. Bayesian analysis of the mitochondrial data (*COI* and *mt16S* concatenated) confirmed that $\mathrm{clamA}_{\mathrm{VII}}$ and $\mathrm{clamB}_{\mathrm{VII}}$ clustered in a monophyletic clade with the clam lineages *Vesicomya* sp. mt-I and -III from the eastern Pacific (PP = 0.97; fig. 1A).

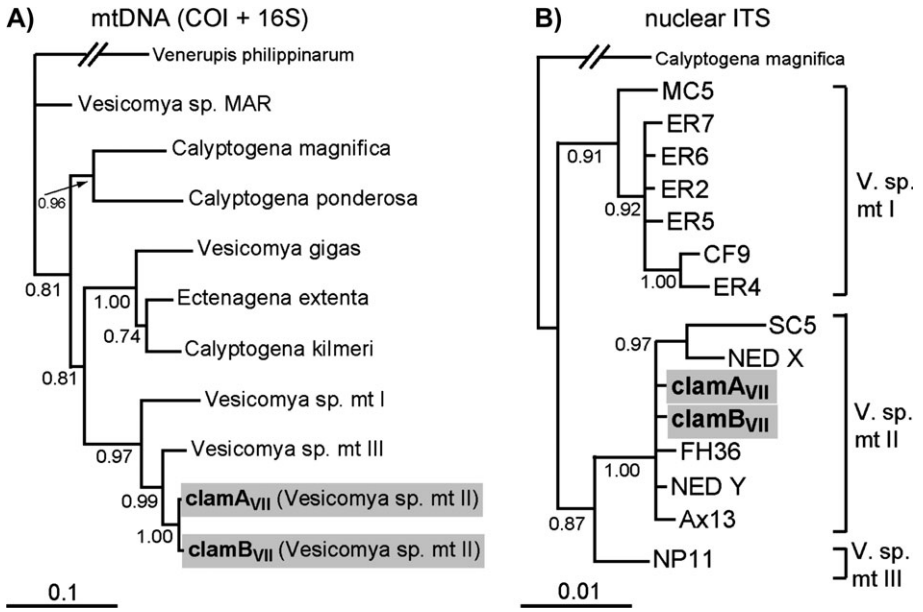

FIG. 1.—Bayesian phylogenies for host loci of vesicomyid clams. PPs of taxon bipartitions are displayed if >0.50. Host trees based on (*A*) the mitochondrial *COI* and *16S* (*mt16S*) genes (concatenated) from clams sequenced in this study, where taxa are named according to top blast hits (>99% nucleotide identity) and (*B*) the nuclear ITS region (*nucITS*) from host clams clamA$_{VII}$ and clamB$_{VII}$ sequenced in this study and clams of the *Vesicomya* sp. mt-I, -II, and -III lineages that were sequenced and named in Goffredi et al. (2003). Host clam haplotypes clamA$_{VII}$ and clamB$_{VII}$ are shaded and belong to the *Vesicomya* sp. mt-II lineage.

The relationship of *nucITS* sequences from clamA$_{VII}$ and clamB$_{VII}$ to those from *Vesicomya* sp. mt-I–, mt-II–, and mt-III–type clams characterized by Goffredi et al. (2003) is shown in figure 1B. ClamA$_{VII}$ and clamB$_{VII}$ were of the same host lineage for this nuclear gene and were identical to a previously sequenced *Vesicomya* sp. mt-II clam.

Symbiont Phylogeny

Symbiont genetic diversity in the 1991 JdF clams was initially assessed by direct sequencing of the bacterial 16S rRNA gene (*16S*; 1,303 bp). Genomic analyses indicate that the rRNA operon occurs in a single copy in vesicomyid symbionts (Kuwahara et al. 2007; Newton et al. 2007), and the chromatograms in our study showed no evidence of a mixed DNA template during direct sequencing. The symbiont *16S* sequence from hosts bearing haplotype clamA$_{VII}$ belonged to the *Vesicomya* sp. mt-II symbiont lineage, differing from the published sequence (see Peek et al. 1998) at only 1 nucleotide (0.08% divergence); we designate this symbiont phylotype symA$_{VII}$. In contrast, the *16S* of the individual bearing haplotype clamB$_{VII}$ was highly divergent, differing from symA$_{VII}$ at 14 sites (1.1%; supplementary table 5, Supplementary Material online); we designate this divergent symbiont phylotype symB$_{VII}$. The level of *16S* divergence of symB$_{VII}$ fell within the range shown for symbionts of distinct vesicomyid host species and contrasted with prior results showing zero symbiont *16S* variation among hosts of the same species (Peek et al. 1998). A BlastN search of the symB$_{VII}$ *16S* sequence returned a top hit not to the *Vesicomya* sp. mt-II symbiont (symA$_{VII}$) but to the symbiont of a vesicomyid (*Calyptogena phaseoliformis*) from the Japan Trench in the western Pacific (99% identity).

Bayesian phylogenetic analysis of *16S* sequences from a wide range of chemosynthetic symbionts and free-living bacteria showed that the *16S* of symB$_{VII}$ fell in the monophyletic clade of vesicomyid symbionts with PP = 1.00 (fig. 2). Within this clade, the placement of the symB$_{VII}$ *16S* sequence is poorly resolved, as this sequence appears to have accumulated few changes relative to that of other vesicomyid symbionts (fig. 3). The *16S* analysis also failed to resolve (PP < 0.5) relationships among several of the other symbiont taxa, particularly those within the clade containing *Vesicomya* sp. mt-I, -II, -III and *C. ponderosa* symbionts (figs. 2 and 3).

The unresolved placement of symB$_{VII}$ in the *16S* tree prompted analyses of 3 additional symbiont genes, *23S*, *dnaK*, and *soxA* (fig. 3). In contrast to that of the *16S*, analysis of the *23S* rRNA gene, which occurs in the same operon as the *16S* gene, yielded a highly resolved vesicomyid symbiont phylogeny, with high PPs at most nodes. This analysis unambiguously placed symB$_{VII}$ as sister to the symbiont of an unnamed clam from the Logatchev vent site on the Mid-Atlantic Ridge, *Vesicomya* sp. MAR (PP = 1.00; also see Peek et al. (2000) for a characterization of the host *COI* of *Vesicomya* sp. MAR). Analysis of the housekeeping chaperone gene *dnaK* and the sulfur oxidation gene *soxA* confirmed the coupling of symB$_{VII}$ and the *Vesicomya* sp. MAR symbiont, with PPs of 1.00 and 0.97, respectively (fig. 3). Nucleotide identities between symB$_{VII}$ and *Vesicomya* sp. MAR symbionts for *dnaK* and *soxA* were 98.8% and 99.0%, respectively (supplementary table 5, Supplementary Material online). In contrast, the median pairwise nucleotide identity across all the symbionts in figure 3 was 90.2% for *soxA* and 86.5% for *dnaK*.

The discovery of symB$_{VII}$ led us to examine the frequency at which this phylotype occurs in other individuals.

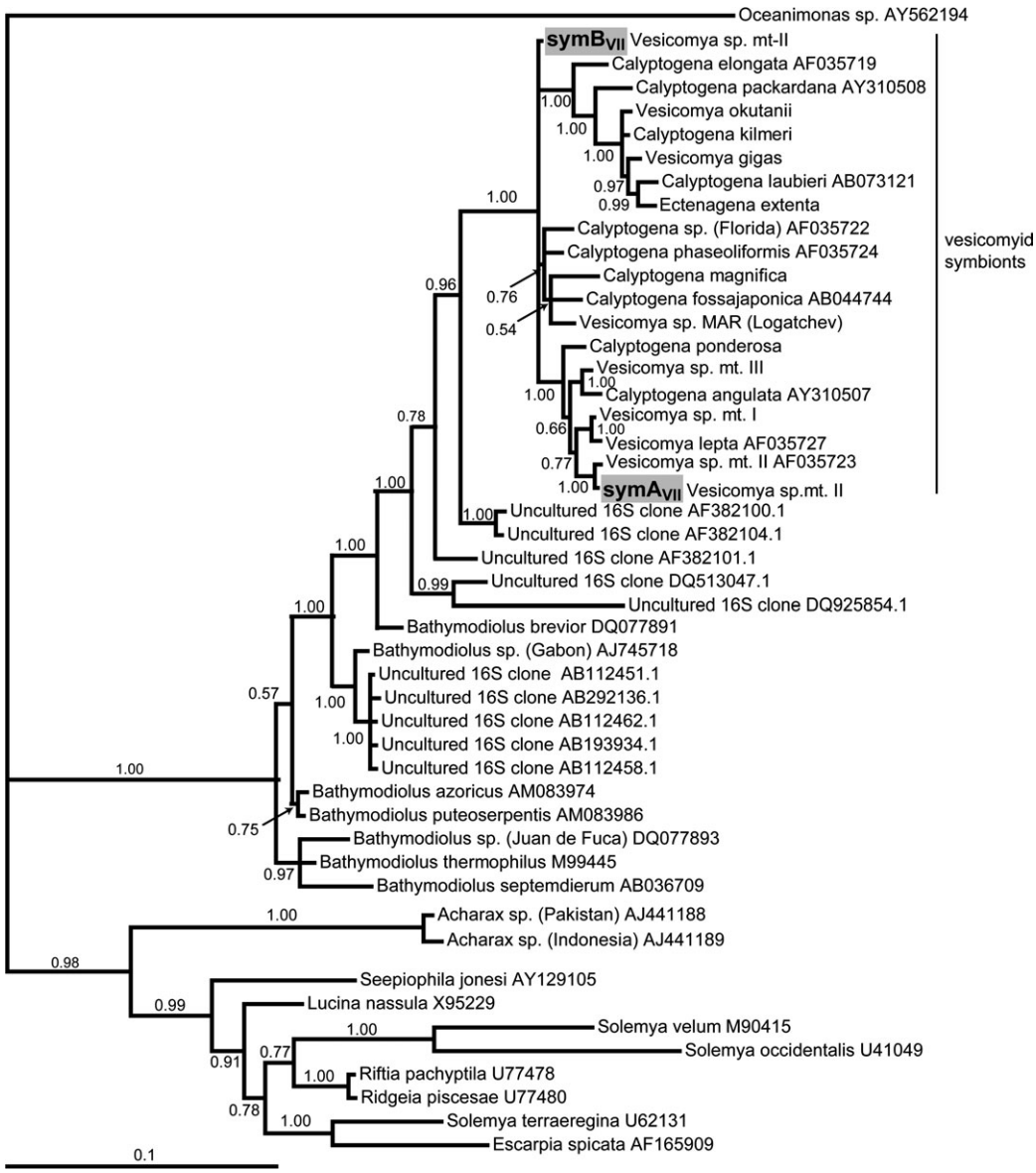

FIG. 2.—Bayesian phylogeny for bacterial 16S rRNA genes from chemosynthetic vesicomyid clam symbionts, symbionts of other marine invertebrates, and closely related free-living bacteria and environmental clones (as identified by BlastN of the $symB_{VII}$ *16S* sequence). PPs of taxon bipartitions are displayed if >0.50. Symbionts are identified according to host taxon. Those sequences not obtained in this study are identified by GenBank accession numbers. Symbiont phylotypes $symA_{VII}$ and $symB_{VII}$ from *Vesicomya* sp. mt-II clams are shaded.

To do so, we sequenced the symbiont *16S* from 115 additional clams collected from the JdF Ridge in 1995 and 1999 (table 1). The $symB_{VII}$ phylotype was detected in a single clam from the 1999 collection (Alvin dive 3459). Additional sequencing of *23S*, *dnaK*, and *soxA* confirmed that the symbiont from this clam was identical to the divergent $symB_{VII}$ phylotype originally detected in the 1991 clam. Similarly, the host loci (*COI*, *mt16S*, *nucITS*) from this individual were identical to those of $clamB_{VII}$ (i.e., correspond to a *Vesicomya* sp. mt-II clam). The *16S* sequences from the remaining 114 clams collected in 1995 and 1999 were identical to the *16S* of $symA_{VII}$, the phylotype previously characterized for the *Vesicomya* sp. mt-II host lineage. The frequency of $symB_{VII}$-hosting clams in the sampled populations was therefore 2 in 118 (0.02).

Upon first finding $symB_{VII}$ in the original 1991 JdF clam, we took several measures to rule out the possibility that this sequence represents a contaminant (i.e., a free-living bacterium associated with the gill). These methods are detailed in Supplementary Material online. However, the question of contamination was effectively nullified by finding $symB_{VII}$ in a second clam from a distinct population (JdF 1999), as well as by our multilocus characterization that unambiguously placed $symB_{VII}$ as sister to another vesicomyid symbiont.

Tests for Symbiont–Host Coupling

The phylogenetic placement of $symB_{VII}$ with the symbiont of a distantly related host clam (*Vesicomya* sp. MAR)

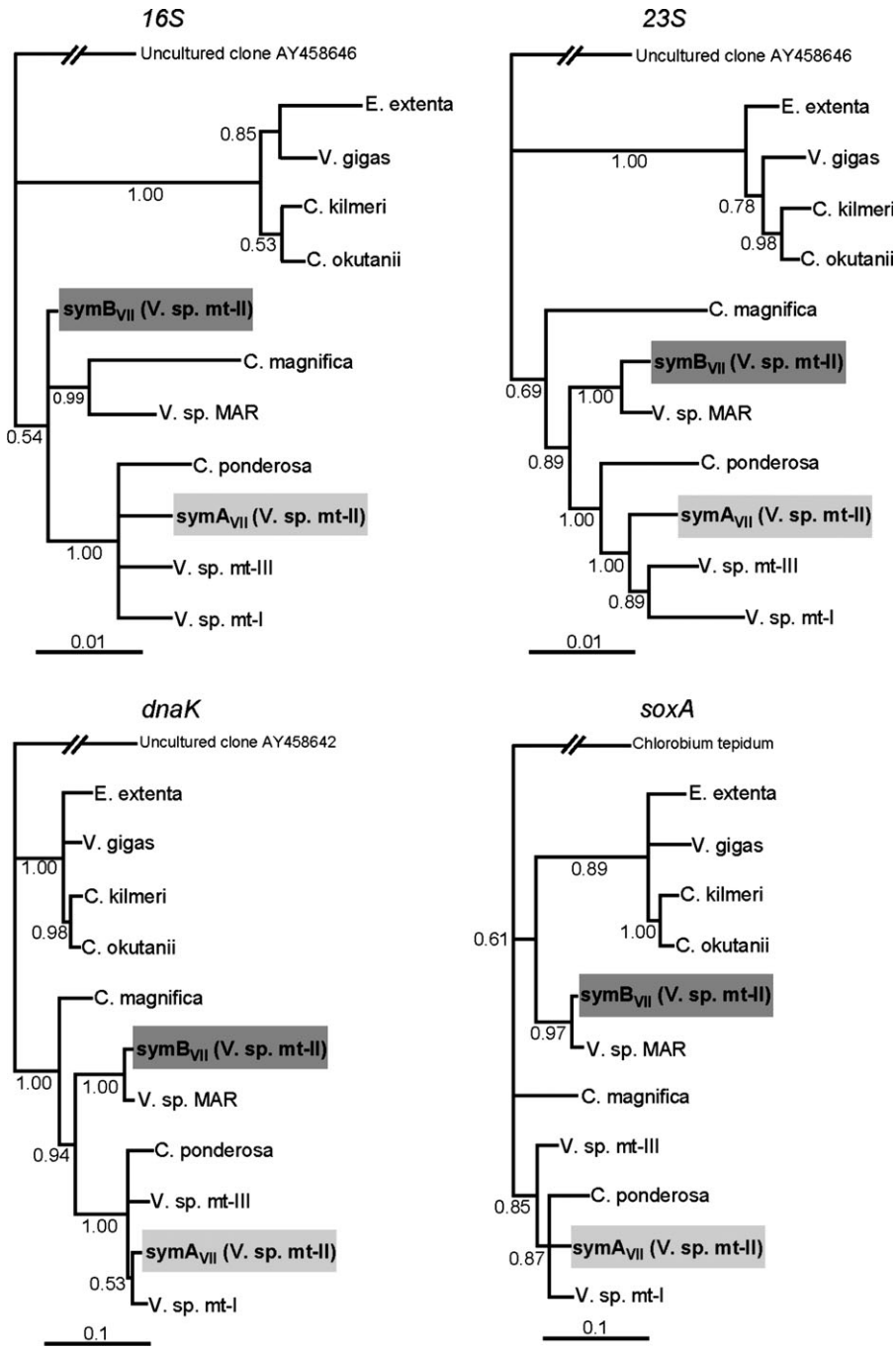

FIG. 3.—Rooted Bayesian phylogenies for symbiont 16S rRNA, 23S rRNA, *dnaK*, and *soxA* genes from 11 vesicomyid clam taxa. PPs of taxon bipartitions are displayed if >0.50. Symbiont phylotypes $symA_{VII}$ and $symB_{VII}$ from *Vesicomya* sp. mt-II clams are shaded. Substitution rates in protein-coding loci are approximately 10× rates in rRNA genes (note differences in scale bars). Symbionts are identified according to host clam. Outgroups were chosen for each locus using BlastN to identify the most closely related free-living bacterium. Relative to *16S* sequences, sequences of bacterial *23S*, *dnaK*, and *soxA* genes are not well represented in GenBank. As such, choice of outgroup for these genes was constrained by availability, with the outgroup coming from whole genomes or large environmental contigs and generally falling outside of the extended *16S* phylogeny shown in figure 2.

indicates a clear decoupling of symbiont and host mitochondrial data. To statistically test for symbiont–host coupling, the symbiont and host mitochondrial data were concatenated and a coupling model in which symbiont and host loci share the same topology, T_1, was compared with a decoupling model in which symbiont and host loci are allowed to have separate topologies, T_2. As shown by BF and Akaike weights (w_i) computed using AICM, analyses of all 4 of the symbiont genes significantly rejected a coupling of symbiont and host topologies (table 2). Indeed, for each test, the decoupling model T_2 was >1,000 times more likely than the coupled topology model (see Akaike weights in table 2). BIC and likelihood-based AU tests additionally confirmed this decoupling, showing for each symbiont gene a reciprocal rejection of symbiont and host mitochondrial topologies (table 3). Curiously, the

Table 3
Reciprocal Tests for Congruence between ML Topologies of Symbiont and Host Loci

Comparison[a]		Diff-lnL[b]		AU[c]		BIC[d]	
Locus 1	Locus 2	$H_o = 2$	$H_o = 1$	$H_o = 2$	$H_o = 1$	$H_o = 2$	$H_o = 1$
w/ sym/clamB$_{VII}$							
COI	*mt16S*	36.2	4.2	0.001	0.243	$\mathbf{2 \times 10^{-16}}$	**0.014**
COI + *mt16S*	*16S*	151.5	47.7	$\mathbf{3 \times 10^{-82}}$	**0.003**	$\mathbf{2 \times 10^{-66}}$	$\mathbf{2 \times 10^{-21}}$
	23S	176.6	64.8	$\mathbf{2 \times 10^{-79}}$	$\mathbf{6 \times 10^{-68}}$	$\mathbf{2 \times 10^{-77}}$	$\mathbf{8 \times 10^{-29}}$
	dnaK	180.2	218.9	$\mathbf{7 \times 10^{-114}}$	$\mathbf{8 \times 10^{-57}}$	$\mathbf{5 \times 10^{-79}}$	$\mathbf{8 \times 10^{-96}}$
	soxA	230.7	126.4	$\mathbf{7 \times 10^{-40}}$	$\mathbf{8 \times 10^{-05}}$	$\mathbf{7 \times 10^{-101}}$	$\mathbf{1 \times 10^{-55}}$
w/o sym/clamB$_{VII}$							
COI	*mt16S*	15.3	4.2	0.047	0.190	$\mathbf{2 \times 10^{-07}}$	**0.011**
COI + *mt16S*	*16S*	13.2	16.9	0.257	0.042	$\mathbf{2 \times 10^{-06}}$	$\mathbf{4 \times 10^{-08}}$
	23S	16.9	10.5	0.084	0.042	$\mathbf{5 \times 10^{-08}}$	$\mathbf{3 \times 10^{-05}}$
	dnaK	54.7	68.8	$\mathbf{4 \times 10^{-07}}$	$\mathbf{4 \times 10^{-06}}$	$\mathbf{2 \times 10^{-24}}$	$\mathbf{1 \times 10^{-30}}$
	soxA	59.1	23.1	**0.002**	**0.004**	$\mathbf{2 \times 10^{-26}}$	$\mathbf{9 \times 10^{-11}}$

[a] First column for each test is for locus 1 data constrained by the ML topology for locus 2 data ($H_o = 2$); second column for each test is for locus 2 data constrained by the ML topology for locus 1 ($H_o = 1$); bold indicates instances of reciprocal rejection of topological congruence ($P < 0.05$).

[b] Diff-lnL = difference in log-likelihood between constrained and unconstrained topologies.

[c] AU: *P* value of the AU test calculated from the multiscale bootstrap; *P* value = probability of obtaining a more extreme test statistic (diff-lnL) by chance under the null hypothesis of no difference between topologies.

[d] BIC: PP calculated by the BIC approximation.

Bayesian methods (BF, AICM, and BIC) also suggested that the topologies of the 2 host mitochondrial loci, *COI* and *mt16S*, may be decoupled (table 3 and supplementary table 6, Supplementary Material online). However, this pattern was not well supported by the AU tests, which failed to reciprocally reject incongruence between these loci (table 3).

To determine whether symB$_{VII}$ provided the only information in the data rejecting symbiont–host coupling, we repeated the analysis excluding clamB$_{VII}$ and symB$_{VII}$. Even without these sequences, the decoupling model T_2 was >1,000 times more likely than the coupling model for each symbiont gene tested, as assessed via BF and AICM (table 2). Similarly, BIC PPs showed a reciprocal rejection of symbiont and host topologies in the absence of clamB$_{VII}$ and symB$_{VII}$ (table 3). The AU tests also provided evidence of decoupling, though congruence was reciprocally rejected for only 2 of the 4 symbiont loci (*dnaK* and *soxA*). Clearly, taxa other than symB$_{VII}$ contributed to the decoupling of symbiont and host phylogenies, as demonstrated by the unrooted symbiont and host mitochondrial topologies shown in figure 4. Notably, the mitochondrial data provided strong support (PP > 0.98) for the sister grouping of {*C. ponderosa* with *C. magnifica*} as well as {V. sp. mt-II and -III}, whereas the symbiont data grouped the symbionts of {*C. ponderosa*, V. sp. mt-I, -II, and -III} and {V. sp. mt-I and -III} (PP = 1.0 and 0.88, respectively; fig. 4). Together, the data showed that the symbiont phylogeny is incompatible with that of the host, a clear violation of the pattern predicted if symbionts and host mitochondria have undergone strict maternal cotransmission throughout the evolutionary history of this group.

Discussion

Symbiont–Host Decoupling Suggests Lateral Symbiont Acquisition

The results of this study suggest that symbionts, or mitochondria, have been transferred laterally (nonvertically) among hosts during the evolutionary history of vesicomyids. This study, interpreted alongside previous studies of the vesicomyid symbioses (Peek et al. 1998, Hurtado et al. 2003), suggests a mixed mode of symbiont transmission characterized by predominantly vertical transmission punctuated with instances of lateral symbiont acquisition. This conclusion is supported by two primary lines of evidence that reject the pattern of genetic coupling predicted if symbionts and mitochondria are strictly maternally cotransmitted (see Moran et al. 1993; Peek et al. 1998; Clark et al. 2000).

First, this study provides the first report of 2 similar symbiont lineages, symB$_{VII}$ and the *Vesicomya* sp. MAR symbiont, occurring in distantly related hosts. SymB$_{VII}$ co-occurs with the previously characterized symbiont phylotype, symA$_{VII}$ (Peek et al. 1998; Goffredi et al. 2003), in clams of the *Vesicomya* sp. mt-II host lineage from the JdF Ridge. However, symB$_{VII}$ is highly divergent from symA$_{VII}$ across rRNA and protein-coding genes, clustering instead with the symbiont of a distinct host lineage, *Vesicomya* sp. MAR from the Logatchev vent field on the Mid-Atlantic Ridge (fig. 3). Given the geographical isolation of the JdF (northeastern Pacific) from the Logatchev site (equatorial Atlantic), the grouping of symB$_{VII}$ and the MAR symbiont might seem curious. However, clustering of Atlantic and Pacific species has been shown repeatedly in vesicomyids (Peek et al. 1997, 1998, 2000; Kojima et al. 2004). Prior studies show the *Vesicomya* sp. MAR (Logatchev) host falling in a clade with clams from the northern Pacific, including unnamed species from the Aleutian Trench, *C. kakoi* from the Nankai Trough off the coast of Japan, and *C. phaseoliformis* from the Japan Trench (Peek et al. 2000; Kojima et al. 2004). Indeed, a BlastN search of the *sym16S* sequence of symB$_{VII}$ returns a top hit to the symbiont of *C. phaseoliformis*. It therefore seems likely that symB$_{VII}$ shares a most recent common ancestor with the symbiont of an as-yet unidentified Pacific clam, which may cluster within the {V. sp. MAR, *C. phaseoliformis*} clade. The current study was constrained

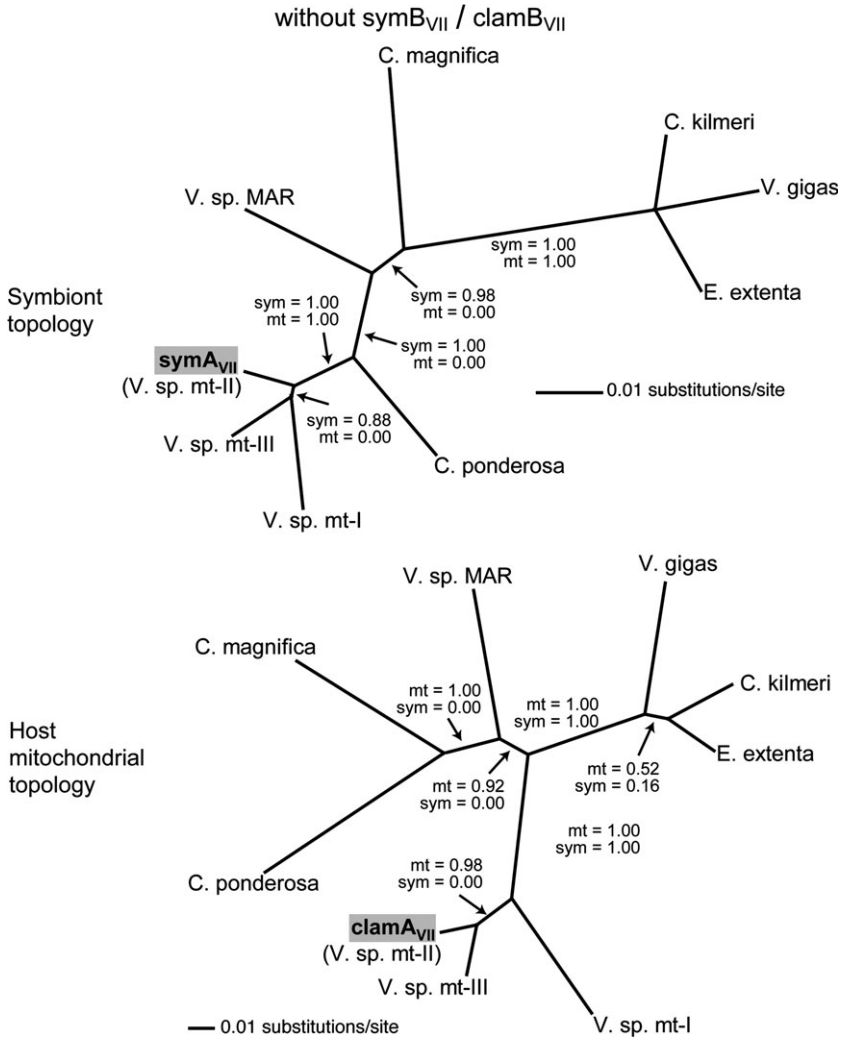

FIG. 4.—Unrooted Bayesian phylogenies for the analysis of 4 concatenated symbiont loci (*16S, 23S*, *dnaK*, and *soxA*) and 2 concatenated host mitochondrial loci (*COI* and *mt16S*) from 9 vesicomyid clam taxa. The divergent symbiont symB$_{VII}$ and its corresponding host haplotype (clamB$_{VII}$) are excluded. Symbiont phylotype symA$_{VII}$ and host haplotype clamA$_{VII}$ (from a *Vesicomya* sp. mt-II clam) are shaded. PPs of taxon bipartitions for both the symbiont and the mitochondrial data are shown on each topology to illustrate incongruities between the data sets. Symbionts are identified according to host clam.

to the available samples; broader taxonomic sampling may reveal the source clam or population from which symB$_{VII}$ originated. Nonetheless, the clustering of symB$_{VII}$ and MAR shown here represents a clear uncoupling of symbiont and host phylogenies, suggesting lateral transfer of symB$_{VII}$ into the *Vesicomya* sp. mt-II host lineage.

Second, Bayesian model discrimination methods (AICM, BF, BIC) show that even in the absence of the divergent symbiont lineage symB$_{VII}$, the symbiont phylogeny is incompatible with that of the host (tables 2 and 3 and fig. 4). This decoupling is further supported for 2 symbiont genes by the likelihood-based AU test (table 3). These results are inconsistent with prior studies. Using a different set of taxa, Peek et al. (1998) showed that the clam mitochondrial topology was not significantly worse than the symbiont *16S* topology at explaining symbiont diversification. This pattern was taken as strong evidence of cospeciation and of nearly complete vertical symbiont transmission in vesicomyids, though the authors hypothesize that minor discrepancies at 2 of the 7 internal nodes in their topologies might be explained by rare lateral symbiont transfer events. Subsequently, Hurtado et al. (2003) examined 80 individuals from populations of the clam *C. magnifica* and found no evidence of a decoupling of distinct symbiont and mitochondrial variants, as might be expected if occasional lateral symbiont transfer occurs between hosts. Together, these studies have led to a general acceptance of the hypothesis that vesicomyid symbionts undergo complete, or nearly complete, maternal transmission (e.g., see review by Papke and Ward 2004). However, detecting symbiont–host coupling likely depends on the taxa included in the study and on the resolution provided by the sequence data (Page 2003). For instance, our study included *Calyptogena ponderosa*, a taxon for which symbiont–host codivergence had not been previously assessed and whose phylogenetic placement in this study appears inconsistent between symbiont and host gene trees (fig. 4). Further, our study utilized a relatively large amount of symbiont sequence data (~5 kb

per taxon), yielding resolution beyond that provided by traditional analyses of the bacterial 16S rRNA gene.

The evidence outlined above argues against the strict maternal cotransmission of vesicomyid symbionts and host mitochondria. Rather, it appears that vesicomyid symbionts, though predominantly vertically transmitted (Peek et al. 1998; Hurtado et al. 2003), also experience occasional instances of lateral symbiont acquisition, with $symB_{VII}$ being the most obvious example. This mixed transmission strategy may be similar to that observed in associations between arthropods and the bacterium *Wolbachia*. As in this study, lateral transfer of cytoplasmically inherited *Wolbachia* has been inferred from the detection of highly similar *Wolbachia* strains in divergent host species, strong incongruence between symbiont and host phylogenies, and evidence of genomic exchange (recombination) between symbiont lineages (Werren et al. 1995; Schilthuizen and Stouthamer 1997; Haine et al. 2005; Baldo et al. 2006). For vesicomyids, the strong phylogenetic signal for symbiont–host codiversification shown in prior studies suggests that, relative to *Wolbachia*, lateral transfer events in vesicomyids may be relatively rare. Clearly, studies aimed at determining the modes and rates of lateral symbiont acquisition for this group are warranted. It is possible that different host lineages (or conversely, symbiont lineages) within the Vesicomyidae are differentially capable of lateral transmission. We now propose several hypothetical mechanisms by which lateral symbiont transfer could occur in vesicomyids (fig. 5). These include acquisition via 1) host hybridization, 2) horizontal transfer, and 3) environmental acquisition. We examine evidence for and against each of these hypotheses below, focusing explicitly on the acquisition event by which $symB_{VII}$ entered the *Vesicomya* sp. mt-II host lineage.

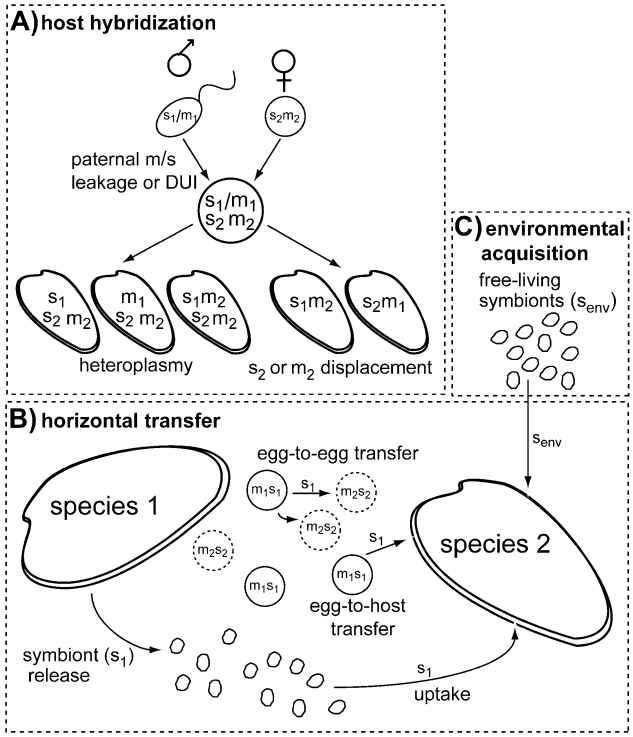

FIG. 5.—Hypothetical mechanisms of lateral (nonvertical) symbiont acquisition in vesicomyid clam symbioses. (*A*) Host hybridization hypothesis. Symbionts (s) and/or mitochondria (m) are transmitted paternally via the sperm during hybridization between species 1 and 2. Paternal transmission may be occasional (leakage) or persistent via DUI, though neither has not been shown for vesicomyids. Potential outcomes of hybridization include the occurrence of multiple symbiont or mitochondrial lineages in the same host (heteroplasmy), or displacement, in which the paternal symbiont or mitochondrial lineage replaces the maternal lineage. (*B*) Horizontal transfer hypothesis. Symbionts are transmitted between contemporary species without hybridization. Potential transfer mechanisms include direct contact between symbiont-associated eggs, between eggs and host tissue (e.g., gill), and between host tissue and symbionts that have been released into the water column. (*C*) Environmental acquisition hypothesis. Symbionts are acquired directly from a stable free-living population of cells. Symbiont acquisition may involve displacement of the existing maternally transferred symbiont lineage, as multiple phylotypes have not been documented within the same host.

Potential Mechanisms of Lateral Symbiont Acquisition

Host Hybridization

$SymB_{VII}$ could have originated from a hybridization event between *Vesicomya* sp. mt-II and an as-yet unidentified clam. This hypothesis would necessarily involve paternal transfer of either symbionts or mitochondria via the sperm (fig. 5A). Such an event presumably would have been followed by displacement or loss of the existing symbiont or mitochondrial genome of the egg, as our sequence traces (and the results of prior studies; Kim et al. 1995; Peek et al. 1998; Goffredi et al. 2003) provide no evidence of multiple symbiont or mitochondrial phylotypes in the same host individual, that is, heteroplasmy. Several lines of evidence argue against a hybridization hypothesis. First, paternal transfer of either mitochondria or symbionts would be necessary to explain the incongruence between mitochondrial and symbiont genealogies observed for the vesicomyids in our study. Paternal symbiont transfer has not been reported in these clams, though this hypothesis has not been rigorously tested. Similarly, paternal mitochondrial transfer, via either occasional leakage (e.g., Kvist et al. 2003; Sherengul et al. 2006) or doubly uniparental inheritance (DUI, Zouros et al. 1994), has not been reported in vesicomyids. However, DUI has been identified in the venerid clam *Tapes philippinarum* and may be an ancestral feature of the Bivalvia (Passamonti and Scali 2001), suggesting the need for further study of this mitochondrial transmission mode in other clams. Second, sequences from 2 nuclear loci, *nucITS* (fig. 1) and histone (Hist2h3c1, 300 bp; data not shown), show no variation between host clams $clamA_{VII}$ and $clamB_{VII}$, suggesting that $clamB_{VII}$ is not a hybrid.

Nevertheless, 2 factors suggest that the hybrid acquisition hypothesis deserves further attention. First, analysis of only 2 nuclear loci might not identify hybrids that have backcrossed for a sufficient number of generations. Indeed, introgression between *Vesicomya* sp. mt-II and an as-yet-unknown species might occur at a rate that would be sufficient for occasional horizontal transmission of symbionts but would not create strong disequilibrium between nuclear and cytoplasmic genes within the host lineage. Low rates of genetic introgression have been demonstrated for *Drosophila pseudoobscura* and *Drosophila persimilis* (Hey and

Nielsen 2004), subspecies of chimpanzees (Won and Hey 2005), cichlid fishes (Hey et al. 2004), *Heliconius* butterflies (Bull et al. 2006), and possibly hydrothermal vent limpets (Johnson et al. 2006). Second, our results show topological incongruence between the 2 host mitochondrial loci used in the analysis (*COI* and *mt16S*; tables 2 and 3). Similarly, a low-level of *COI–mt16S* incongruence was demonstrated previously for some vesicomyid taxa (Peek et al. 2000). Such incongruence is suggestive of recombination, though other factors, including inadequate substitution models, may contribute to this pattern (Peek et al. 2000). If low levels of introgression occur between *Vesicomya* sp. mt-II and another species, then multilocus screening of host genes should also show introgression between the species. Clearly, analysis of additional nuclear loci, as well as broader taxonomic sampling of vesicomyid hosts and symbionts, is necessary to verify or rule out the hybridization hypothesis. However, the data currently available suggest that $symB_{VII}$ did not originate via hybridization.

Horizontal Transfer

$SymB_{VII}$ could have originated via horizontal host-to-host transfer that did not involve hybridization (fig. 5B). Horizontal transfer of cytoplasmically inherited endosymbionts has been observed repeatedly in associations between arthropods and the parasitic bacterium *Wolbachia* (e.g., Haine et al. 2005). The mechanism of *Wolbachia* transfer in nature is poorly understood but may involve shared interactions with common food substrates, predators, or parasites (Huigens et al. 2004; Sintupachee et al. 2006). In vesicomyid clams, a horizontal transfer hypothesis would involve both symbiont release from the donor clam and acquisition by the recipient species (*Vesicomya* sp. mt-II; fig. 5B). As in the host hybridization hypothesis, symbiont uptake would likely be followed by displacement of the existing symbiont population. Symbiont release could occur via attachment to the egg, as vesicomyid symbionts have been shown to occur at high density in the nutritive follicle cells surrounding the primary oocyte of the clam (Cary and Giovannoni 1993). Alternatively, symbionts could be released directly into the environment, perhaps from a dead or moribund clam, though there is no evidence to suggest this mechanism. Acquisition by the recipient clam would then involve contact with either egg-associated or freely dispersing symbionts, followed by symbiont internalization into the tissue of the new host. One could imagine this process occurring at the gamete stage if eggs from distinct species come into physical contact, thereby enabling symbiont exchange. Several aspects of this hypothesis, including the possibility of egg-to-egg transfer, may be experimentally testable and require further attention.

Environmental Acquisition

$SymB_{VII}$ could have been acquired directly from the environment from a free-living pool of symbionts (fig. 5C). Environmental acquisition has been suggested in other vent chemosynthetic symbioses, including mytilid mussels and vestimentiferan tube worms (Won et al. 2003; DeChaine et al. 2006; Nussbaumer et al. 2006; Vrijenhoek et al. 2007). Environmental acquisition in these taxa has been inferred from molecular evidence showing multiple symbiont phylotypes within a single host individual, as would be expected if symbionts are acquired from a diverse external population. Other studies of these taxa have used experimental or cytological data to directly show symbiont internalization or acquisition by the host (Kadar et al. 2005; Nussbaumer et al. 2006). However, similar evidence has not been reported for vesicomyids, and vesicomyid symbionts have not been found free living in the environment, though intensive searches for these bacteria in a free-living form have not been conducted. Further, accelerated substitution rates in vesicomyid symbionts are consistent with a maternal transmission strategy that inhibits gene exchange with a free-living bacterial pool (Peek, Vrijenhoek, and Gaut 1998). Recent genome sequencing of 2 vesicomyid symbionts suggests that the symbiont genome ($\sim$1.1 Mb) has experienced some level of size reduction (Kuwahara et al. 2007; Newton et al. 2007). Genome reduction is well documented in other obligately endosymbiotic bacteria, including the gut bacteria of insects (size range: $\sim$0.42–0.65 Mb; Pérez-Brocal et al. 2006; Gómez-Valero et al. 2007). Although larger than that of other endosymbionts, the reduced genome of vesicomyid symbionts nonetheless may indicate adaptation to the intracellular environment. Such adaptation may inhibit growth outside the host, though this hypothesis requires further testing. Nevertheless, the available data argue against long-term persistence of a free-living vesicomyid symbiont pool.

Implications of Lateral Symbiont Acquisition in Vesicomyids

This study provides compelling evidence that $symB_{VII}$ was acquired laterally (i.e., nonmaternally). However, additional analyses, as well as broader taxonomic sampling, are necessary to address the mechanisms by which this could have occurred. Of the above hypotheses, environmental acquisition may be the most difficult to test experimentally, as definitive proof of this hypothesis would require recurring detection of vesicomyid symbionts in their free-living form. Hypotheses regarding acquisition via host hybridization or horizontal host-to-host transfer may warrant particular attention, as aspects of these hypotheses (e.g., paternal symbiont or mitochondrial transfer, egg-to-egg transfer) can be tested experimentally. Additional studies should also examine the temporal scale and frequency of lateral transfer. The presence of $symB_{VII}$ in *Vesicomya* sp. mt-II most likely resulted from a single lateral transfer event, followed by maintenance of $symB_{VII}$ at low frequency via vertical transmission. This is supported by the fact that the two clams hosting $symB_{VII}$ have exactly the same mitochondrial and symbiont haplotypes, suggesting a tight coupling of symbiont and mitochondrial genomes. Nonetheless, we cannot exclude the possibility that $symB_{VII}$ symbionts are being actively transferred into the *Vesicomya* sp. mt-II host lineage at low frequency, and the occurrence of this symbiont phylotype in two clams represents 2 lateral transfer events. Additional population-level sampling of symbiont and host mitochondrial and nuclear

variation may resolve these 2 scenarios. Our results should encourage such studies, as lateral symbiont acquisition has important implications for understanding the evolution of vesicomyid symbioses.

Lateral symbiont acquisition may disrupt coadapted symbiont–host gene interactions. For most vesicomyids, vertical transmission has likely maintained a tight association between a clam and its symbiont over millions of years of evolution. Tight coevolution of this sort may impose severe constraints on the specificity of the symbiont–host interaction. For example, Edmands and Burton (1999) show that in hybrid lineages of *Tigriopus* copepods, introgression of the nuclear genotype from 1 population onto the cytoplasmic (mitochondrial) genotype of a divergent population causes reduced fitness and loss of function of major mitochondrial enzyme complexes. Hybridization presumably disrupts the strong positive epistatic interactions that normally occur between coadapted mitochondrial and nuclear gene products in these complexes (Ellison and Burton 2006). Similar molecular or physiological coadaptations may exist between the vesicomyid symbiont genome and the mitochondrial or nuclear genome of its host, particularly given the extent to which the clam host relies on its symbiont for nutrition (Fiala-Médioni et al. 1993). Our results are therefore notable, as they indicate that at least 1 host lineage (*Vesicomya* sp. mt-II) retains the ability to acquire divergent symbionts. This observation indicates that the specificity of the symbiont–host interaction is not absolute and suggests the possibility that a core set of genes may be conserved across symbiont lineages, thereby allowing the infection of hosts with nonspecific symbiont lineages. Indeed, recent analyses reveal high levels of synteny and gene content conservation in the genomes of the distantly related symbionts of *C. okutannii* and *C. magnifica* (Kuwahara et al. 2007; Newton et al. 2007; Newton ILG, Cavanaugh CM, unpublished data), perhaps indicating that genome reduction occurred prior to large-scale diversification in these symbioses. Additional experimental and genomic studies should focus on the extent to which genome structure and content impact the coadapted symbiont–clam interaction.

Lateral symbiont transmission may also create opportunities for the exchange of genomic material (recombination) between divergent symbiont strains (Baldo et al. 2006). Indeed, the intracellular insect parasite *Wolbachia*, which undergoes maternal transmission as well as occasional instances of lateral transmission (e.g., Haine et al. 2005), exhibits extensive recombination (Baldo et al. 2006). The detection of recent recombination in vesicomyid symbionts would suggest that certain genes are not functionally constrained by high levels of symbiont–host coadaptation and may be exchanged freely among divergent taxa. Further, recombination could impact genome evolution by attenuating the effects of Muller's ratchet observed in strictly vertically transmitted symbionts (Moran 1996). The impact of recombination, if it occurs, would depend on the rate of gene exchange among lineages. This rate might be low, as contact between divergent strains would likely be limited to instances of lateral symbiont transfer, which are presumably rare. Nonetheless, this study provides evidence that lateral symbiont transmission does occur in vesicomyids, indicating that the coevolutionary history of these clams and their internal symbionts is far less static and far more complex than previously thought.

Supplementary Material

Supplementary figures 1–3, supplementary tables 1–6 and supplementary material are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

We thank Bob Vrijenhoek, Scott Edwards, and 2 anonymous reviewers for their generous and constructive input during the preparation of this manuscript. We also thank Pete Girguis, Zoe McCuddin, Chuck Fisher, and Bob Vrijenhoek for generously providing us with vesicomyid samples. This work was supported by National Science Foundation grants EF-0412205 and OCE-0453901 awarded to C.M.C. and by the Genetics and Genomics Training Program at Harvard University.

Literature Cited

Akaike H. 1974. A new look at the statistical model identification. IEEE Trans Automat Control. 19:716–723.

Baldo L, Bordenstein S, Wernegreen JJ, Werren JH. 2006. Widespread recombination throughout *Wolbachia* genomes. Mol Biol Evol. 23:437–449.

Bull V, Beltran M, Jiggins CD, McMillan WO, Bermingham E, Mallet J. 2006. Polyphyly and gene flow between non-sibling *Heliconius* species. BMC Biol. 4:1–17.

Burnham KP, Anderson DR. 2002. Model selection and multi model inference. A practical information-theoretic approach. New York: Springer.

Cary SC, Giovannoni SJ. 1993. Transovarial inheritance of endosymbiotic bacteria in clams inhabiting deep-sea hydrothermal vents and cold seeps. Proc Natl Acad Sci USA. 90: 5695–5699.

Cavanaugh CM. 1983. Chemoautotrophic bacteria in marine invertebrates from sulfide-rich habitats. Nature. 302:58–61.

Chen X, Li S, Aksoy S. 1999. Concordant evolution of a symbiont with its host insect species: molecular phylogeny of genus *Glossina* and its bacteriome-associated endosymbiont, *Wigglesworthia glossinidia*. J Mol Evol. 48:49–58.

Clark MA, Moran NA, Baumann PB, Wernegreen JJ. 2000. Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*) and pitfalls of testing for phylogenetic congruence. Evolution. 54:517–525.

DeChaine EG, Bates AE, Shank TM, Cavanaugh CM. 2006. Off-axis symbiosis found: characterization and biogeography of bacterial symbionts of *Bathymodiolus* mussels from Lost City hydrothermal vents. Environ Microbiol. 8:1902–1912.

Degnan PH, Lazarus AB, Brock CD, Wernegreen JJ. 2004. Host-symbiont stability and fast evolutionary rates in an ant-bacterium association: cospeciation of *Camponotus* species and their endosymbionts, Candidatus *Blochmannia*. Syst Biol. 53:95–110.

Di Meo CA, Wilbur AE, Holben WE, Feldman RA, Vrijenhoek RC, Cary SC. 2000. Genetic variation among endosymbionts of widely distributed vestimentiferan tubeworms. Appl Environ Microbiol. 66:651–658.

Edmands S, Burton RS. 1999. Cytochrome *c* oxidase activity in interpopulation hybrids of a marine copepod: a test for nuclear-nuclear or nuclear-cytoplasmic coadaptation. Evolution. 53:1972–1978.

Ellison CK, Burton RS. 2006. Disruption of mitochondrial function in interpopulation hybrids of *Tigriopus californicus*. Evolution. 60:1382–1391.

Endow K, Ohta S. 1990. Occurrence of bacteria in the primary oocytes of vesicomyid clam *Calyptogena soyoae*. Mar Ecol Prog Ser. 64:309–311.

Fiala-Médioni A, Métivier C. 1986. Ultrastructure of the gill of the hydrothermal vent bivalve *Calyptogena magnifica*, with a discussion of its nutrition. Mar Biol. 90:215–222.

Fiala-Médioni A, Boulegue J, Ohta S, Felbeck H, Mariotti A. 1993. Source of energy sustaining the *Calyptogena* populations from deep trenches in subduction zones off Japan. Deep-Sea Res. 40(Pt 1):1241–1258.

Friedrich CG, Rother D, Bardischewsky F, Quentmeier A, Fischer J. 2001. Oxidation of reduced inorganic sulfur compounds by bacteria: emergence of a common mechanism. Appl Environ Microbiol. 67:2873–2882.

Gelman A, Carlin JB, Stern HS, Rubin DB. 2004. Bayesian data analysis, 2nd ed. London: Chapman and Hall.

Gómez-Valero L, Silva FJ, Simon JC, Latorre A. 2007. Genome reduction of the aphid endosymbiont *Buchnera aphidicola* in a recent evolutionary time scale. Gene. 389:87–95.

Goffredi SK, Hurtado LA, Hallam S, Vrijenhoek RC. 2003. Evolutionary relationships of deep-sea vent and seep clams (Mollusca: vesicomyidae) of the '*pacifica*/*lepta*' species complex. Mar Biol. 142:311–320.

Gupta R. 1998. Protein phylogenies and signature sequences: a reappraisal of evolutionary relationships among Archaebacteria, Eubacteria, and Eukaryotes. Microbiol Mol Biol Rev. 62:1435–1491.

Haine ER, Pickup NJ, Cook JM. 2005. Horizontal transmission of *Wolbachia* in a *Drosophila* community. Ecol Entomol. 30:464–472.

Hey J, Nielsen R. 2004. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. Genetics. 167:747–760.

Hey J, Won YJ, Sivasundar A, Nielsen R, Markert JA. 2004. Using nuclear haplotypes with microsatellites to study gene flow between recently separated Cichlid species. Mol Ecol. 13:909–919.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics. 17:754–755.

Huigens ME, Almeida RP, de Poons PAH, Luck RF, Stouthamer R. 2004. Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* in *Trichogramma* wasps. Proc R Soc Lond B Biol Sci. 261:509–515.

Hurtado LA, Mateos M, Lutz RA, Vrijenhoek RC. 2003. Coupling of bacterial endosymbiont and host mitochondrial genomes in the hydrothermal vent clam C*alyptogena magnifica*. Appl Environ Microbiol. 69:2058–2064.

Johnson SB, Young CR, Jones WJ, Waren A, Vrijenhoek RC. 2006. Migration, isolation, and speciation of hydrothermal vent limpets (Gastropoda; Lepetodrilidae) across the Blanco transform fault. Biol Bull. 210:140–157.

Kadar E, Bettencourt R, Costa V, Santos RS, Lobo-Da-Cunha A, Dando P. 2005. Experimentally induced endosymbiont loss and re-acquirement in the hydrothermal vent bivalve *Bathymodiolus azoricus*. J Exp Mar Biol Ecol. 318: 99–110.

Kass RE, Raftery AE. 1995. Bayes factors. J Am Stat Assoc. 90:773–795.

Kennish MJ, Lutz RA. 1992. The hydrothermal vent clam, *Calyptogena magnifica* (Boss and Turner, 1980): a review of existing literature. Rev Aquat Sci. 6:29–66.

Kiel S, Little CTS. 2006. Cold-seep mollusks are older than the general marine mollusk fauna. Science. 313:1429–1431.

Kim YW, Yasuda M, Yamagishi A, Oshima T, Ohta S. 1995. Characterization of the endosymbiont of a deep-sea bivalve, *Calyptogena soyae*. Appl Environ Microbiol. 61:823–827.

Kojima S, Fujikura K, Okutani T. 2004. Multiple trans-Pacific migrations of deep-sea vent/seep-endemic bivalves in the family Vesicomyidae. Mol Phylogenet Evol. 32:396–406.

Krueger DM, Cavanaugh CM. 1997. Phylogenetic diversity of bacterial symbionts of *Solemya* hosts based on comparative sequence analysis of 16S rRNA genes. Appl Environ Microbiol. 63:91–98.

Krylova EM, Sahling H. 2006. Recent bivalve molluscs of the genus *Calyptogena* (Vesicomyidae). J Molluscan Stud. 72:359–395.

Kuwahara H, Yoshida T, Takaki Y, et al. (10 co-authors). 2007. Reduced genome of the thioautotrophic intracellular symbiont in a deep-sea clam, *Calyptogena okutanii*. Curr Biol. 17: 881–886.

Kvist L, Martens J, Nazarenko AA, Orell M. 2003. Paternal leakage of mitochondrial DNA in the great tit (*Parus major*). Mol Biol Evol. 20:243–247.

Lartillot N, Philippe H. 2006. Computing Bayes factors using thermodynamic integration. Syst Biol. 55:195–207.

Lo N, Bandi C, Watanabe H, Nalepa C, Beninati T. 2003. Evidence for cocladogenesis between diverse Dictyopteran lineages and their intracellular endosymbionts. Mol Biol Evol. 20:907–913.

Maddison DR, Maddison WP. 2000. MacClade 4: analysis of phylogeny and character evolution. Sunderland (MA): Sinauer Associates.

Moran NA. 1996. Accelerated evolution and Muller's ratchet in endosymbiotic bacteria. Proc Natl Acad Sci USA. 93: 2873–2878.

Moran NA, Munson MA, Baumann P, Ishikawa H. 1993. A molecular clock in endosymbiotic bacteria is calibrated using the insect hosts. Proc R Soc Lond B Biol Sci. 253:167–171.

Nelson K, Fisher C. 2000. Absence of cospeciation in deep-sea vestimentiferan tube worms and their bacterial endosymbionts. Symbiosis. 28:1–15.

Newton MA, Raftery AE. 1994. Approximate Bayesian inference by the weighted likelihood bootstrap. J R Stat Soc B. 56:3–48.

Newton ILG, Woyke T, Auchtung TA, et al. (16 co-authors). 2007. The *Calyptogena magnifica* chemoautotrophic symbiont genome. Science. 315:998–1000.

Nussbaumer AD, Fisher CR, Bright M. 2006. Horizontal endosymbiont transmission in hydrothermal vent tubeworms. Nature. 441:345–348.

Nylander JAA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL. 2004. Bayesian phylogenetic analysis of combined data. Syst Biol. 53:47–67.

Page RDM. 2003. Tangled trees: phylogeny, cospeciation, and coevolution. Chicago (IL): University of Chicago Press.

Papke RT, Ward DM. 2004. The importance of physical isolation to microbial diversification. FEMS Microbiol Ecol. 48:293–303.

Passamonti M, Scali V. 2001. Gender-associated mitochondrial DNA heteroplasmy in the venerid clam *Tapes philippinarum* (Mollusca Bivalvia). Curr Genet. 39:117–124.

Peek AS, Gaut BS, Feldman RA, Barry JP, Kochevar RE, Lutz RA, Vrijenhoek RC. 2000. Neutral and nonneutral mitochondrial genetic variation in deep-sea clams from the family Vesicomyidae. J Mol Evol. 50:141–153.

Peek AS, Gustafson RG, Lutz RA, Vrijenhoek RC. 1997. Evolutionary relationships of deep-sea hydrothermal vent and

cold-water seep clams (Bivalvia: vesicomyidae): results from the mitochondrial cytochrome oxidase subunit I. Mar Biol. 130:151–161.
Peek AS, Feldman RA, Lutz RA, Vrijenhoek RC. 1998. Cospeciation of chemoautotrophic bacteria and deep-sea clams. Proc Natl Acad Sci USA. 95:9962–9966.
Peek AS, Vrijenhoek RC, Gaut BS. 1998. Accelerated evolutionary rate in sulfur-oxidizing endosymbiotic bacteria associated with the mode of symbiont transmission. Mol Biol Evol. 15:1514–1523.
Pérez-Brocal V, Gil R, Ramos S, Lamelas A, Postigo M, Michelena JM, Silva FJ, Moya A, Latorre A. 2006. A small microbial genome: the end of a long symbiotic relationship? Science. 314:312–313.
Posada D, Crandall KA. 2001. Selecting the best fit model of nucleotide substitution. Syst Biol. 50:580–601.
R Development Core Team. 2007. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.
Raftery AE, Newton MA, Satagopan JM, Krivitsky PN. 2007. Estimating the integrated likelihood distribution via posterior simulation using the harmonic mean identity. In: Bernardo JM, Bayarri MJ, Berger JO, Dawid AP, Heckerman D, Smith AFM, West M, editors. Bayesian statistics 8. Oxford (UK): Clarendon Press. p. 1–45.
Schilthuizen M, Stouthamer R. 1997. Horizontal transmission of parthenogenesis-inducing microbes in *Trichogramma* wasps. Proc R Soc Lond B Biol Sci. 264:361–366.
Schwarz G. 1978. Estimating the dimension of a model. Ann Stat. 6:461–464.
Sherengul W, Kondo R, Matsuura ET. 2006. Analysis of paternal transmission of mitochondrial DNA in *Drosophila*. Genes Genet Syst. 81:399–404.
Shimodaira H. 2002. An approximately unbiased test of phylogenetic tree selection. Syst Biol. 51:492–508.
Shimodaira H, Hasegawa M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics. 17:1246–1247.
Sintupachee S, Milne JR, Poonchaisri S, Baimai V, Kittayapong P. 2006. Closely related *Wolbachia* strains within the pumpkin arthropod community and the potential for horizontal transmission via the plant. Microb Ecol. 51:294–301.
Stewart FJ, Newton ILG, Cavanaugh CM. 2005. Chemosynthetic endosymbioses: adaptations to oxic-anoxic interfaces. Trends Microbiol. 13:439–448.
Suzuki Y, Kojima S, Watanabe H, et al. (10 co-authors). 2006. Single host and symbiont lineages of hydrothermal vent gastropods *Ifremeria nautilei* (Provannidae): biogeography and evolution. Mar Ecol Prog Ser. 315:167–175.
Swofford DL. 1998. Paup*. Phylogenetic analysis using parsimony (*and other methods). Sunderland (MA): Sinauer.
Tavaré S. 1986. Some probabilistic and statistical problems on the analysis of DNA sequences. Lect Math Life Sci. 17:57–86.
Thompson JD, Higgins DG, Gibson TJ. 1994. ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673–4680.
van Ham RCHJ, Kamerbeek J, Palacios C, et al. (16 co-authors). 2003. Reductive genome evolution in *Buchnera aphidicola*. Proc Natl Acad Sci USA. 100:581–586.
Vrijenhoek RC, Schutz SJ, Gustafson RG, Lutz RA. 1994. Cryptic species of deep-sea clams (Mollusca, Bivalvia, Vesicomyidae) in hydrothermal vent and cold-seep environments. Deep Sea Res II. 41:1171–1189.
Vrijenhoek RC, Duhaime M, Jones WJ. 2007. Subtype variation among bacterial endosymbionts of tubeworms (Annelida: siboglinidae) from the Gulf of California. Biol Bull. 212:180–184.
Werren JH, Zhang W, Guo LR. 1995. Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. Proc R Soc Lond B Biol Sci. 261:55–71.
Wade MJ. 2007. The co-evolutionary genetics of ecological communities. Nat Rev Genet. 8:185–195.
Won YJ, Hey J. 2005. Divergence population genetics of chimpanzees. Mol Biol Evol. 22:297–307.
Won YJ, Hallam SJ, O'Mullan GD, Pan IL, Buck KR, Vrijenhoek RC. 2003. Environmental acquisition of thiotrophic endosymbionts by deep-sea mussels of the genus *Bathymodiolus*. Appl Environ Microbiol. 69:6785–6792.
Yang Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. J Mol Evol. 39:306–314.
Zouros E, Ball AO, Saavedra C, Freeman KR. 1994. An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus*. Proc Natl Acad Sci USA. 91:7463–7467.

Edward Holmes, Associate Editor

Accepted January 4, 2008