Lateral Symbiont Acquisition in a Maternally Transmitted Chemosynthetic Clam Endosymbiosis

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Deep-sea clams of the family Vesicomyidae live in symbiosis with intracellular chemosynthetic bacteria. These symbioses 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 symbBVII) highly divergent from previously described symbionts of the same host lineage. SymBVII-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 symbBVII within the monophyletic clade of vesicomyid symbionts. Further analysis of 3 symbiont loci (23S, dnaK, and soxA) across 11 vesicomyid taxa unambiguously placed symbBVII 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 symBVII 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 symbBVII being the most conspicuous example. Interpreted alongside previous studies of the vesicomyid symbioses, 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 invertebrate host facilitation to substrates (sulfur or methane, oxygen, carbon dioxide) needed for the chemosynthetic 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 symbionts 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, Dulhaine, 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 examined 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 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 Métrivier 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 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 clams’ 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...
transmission hypothesis, the symbiont and host mitochon-
drial genomes necessarily pass through the same individu-
als and are completely genetically linked (Hurtado et al. 2003). Patterns in symbiont variation should therefore par-
allel 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 be-
tween symbiont 16S rRNA and host mitochondrial gene
phylogenies for 9 vesicomyid taxa, though minor discrep-
ancies 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 symbi-
ont and host genealogies exhibited parallel partitions, sug-
gesting a tight coupling of symbiont and host mitochondrial
genomes. Further, focusing on intraspecies and intrapopu-
lation variation, Hurtado et al. (2003) demonstrated symbi-
ton–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 ma-
ternal symbiont transmission in these taxa (Hurtado et al.
2003).

Events that disrupt strict maternal transmission and de-
couple symbiont and clam phylogenies might still be pos-
sible. These include the acquisition of new symbionts from
the environment or the horizontal transfer of symbiont lin-
eages 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 environ-
mental acquisition. A decoupling of symbiont and host phy-
genomes 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 ma-
terial (recombination) between divergent 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 vesico-
myid symbiont and host mitochondrial phylogenies, pro-
viding 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. Fur-
ther, Bayesian analysis using a computationally robust
model choice statistic, the Akaike information criterion
Monte Carlo (AICM; Raftery et al. 2007), demonstrated in-
congruence 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 ad-
tional 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 col-
lection or from collaborators. These samples represent spe-
cies 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), mito-
ochondrial 16S rRNA gene (mt16S; 441 bp), and first internal
transcribed spacer (ITS) of the nuclear rRNA operon (nu-
cITS; 1,190 bp). These host loci have been used previously to
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 23SrRNA
gene (23S; 1,725 bp), and 2 single-copy protein-coding
genes, dnaK and soxA (735 and 594 bp). The soxA gene enc-
odes 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 re-
geons 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 elec-
rophoresis, PCR products were purified using the QIAquick
PCR purification kit (Qiagen). Purified products were di-
rectly sequenced in both the forward and the reverse direc-
tions using standard BigDye techniques. Sequences were
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 symAVIII, whereas the divergent symbiont phylotype described in this study is designated symBVIII. The corresponding host haplotypes are clamAVIII and clamBVIII, respectively. The broad phylogenetic placement of symAVIII and symBVIII 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 symBVIII 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 clamAVIII and clamBVIII host clams, *nuITS* 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, *nuITS* 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 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 (Tavare’ 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 (Akaize 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(1,1,1,1) 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(1,1,1,1,1,1) prior) were shared among the data partitions. Other loci required gamma-distributed rates (a; Uniform(0.05, 50) prior) or a proportion of invariant sites

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**Table 1**

<table>
<thead>
<tr>
<th>Host Species</th>
<th>n</th>
<th>Year</th>
<th>Location</th>
<th>Habitat</th>
<th>Latitude, Longitude</th>
<th>Dive</th>
<th>Depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vesicomya</em> sp. mt-IIb</td>
<td>3</td>
<td>1991</td>
<td>JdF Ridge, North Endeavor</td>
<td>Vent 47-57.4N, 129-05.9W</td>
<td>A 2413</td>
<td>2,200</td>
<td></td>
</tr>
<tr>
<td><em>Vesicomya</em> sp. mt-Ib</td>
<td>32</td>
<td>1995</td>
<td>JdF</td>
<td>Vent 47-58.1N, 129-05.2W</td>
<td>ATV</td>
<td>2,200</td>
<td></td>
</tr>
<tr>
<td><em>Vesicomya</em> sp.</td>
<td>4</td>
<td>1999</td>
<td>JdF</td>
<td>Vent 47-57.78N, 129-05.51W</td>
<td>A 3454</td>
<td>2,182</td>
<td></td>
</tr>
<tr>
<td><em>Vesicomya</em> sp.</td>
<td>6</td>
<td>1999</td>
<td>JdF</td>
<td>Vent 47-57.66N, 129-05.62W</td>
<td>A 3456</td>
<td>2,184</td>
<td></td>
</tr>
<tr>
<td><em>Vesicomya</em> sp.</td>
<td>7</td>
<td>1999</td>
<td>JdF</td>
<td>Vent 47-58.11N, 129-05.24W</td>
<td>A 3457</td>
<td>2,175</td>
<td></td>
</tr>
<tr>
<td><em>Vesicomya</em> sp.</td>
<td>34*</td>
<td>1999</td>
<td>JdF</td>
<td>Vent 47-57.79N, 129-05.48W</td>
<td>A 3459</td>
<td>2,190</td>
<td></td>
</tr>
<tr>
<td><em>Vesicomya</em> sp. MAR</td>
<td>1</td>
<td>2000</td>
<td>Logatchev, Mid-Atlantic Ridge</td>
<td>Vent 45-32N, 44-58.79W</td>
<td>A 3668</td>
<td>3,028</td>
<td></td>
</tr>
<tr>
<td><em>Calyptogena magnifica</em></td>
<td>1</td>
<td>2001</td>
<td>Monterey Canyon</td>
<td>Cold seep 34-66N, 122-51W</td>
<td>5</td>
<td>970</td>
<td></td>
</tr>
<tr>
<td><em>Calyptogena okutanii</em></td>
<td>1</td>
<td>1992</td>
<td>Gulf of Mexico</td>
<td>Cold seep 27-40.88N, 91-32.10W</td>
<td>X</td>
<td>2,200</td>
<td></td>
</tr>
<tr>
<td><em>Calyptogena ponderosa</em></td>
<td>1</td>
<td>1994</td>
<td>Monterey Canyon</td>
<td>Cold seep 34-66N, 122-37W</td>
<td>V</td>
<td>1,694</td>
<td></td>
</tr>
<tr>
<td><em>Ectenogena extenta</em></td>
<td>1</td>
<td>1999</td>
<td>Monterey Bay</td>
<td>Cold seep 34-67N, 122-52W</td>
<td>V</td>
<td>500-1,000</td>
<td></td>
</tr>
<tr>
<td><em>Vesicomya</em> sp. mt-IIIb</td>
<td>1</td>
<td>2000</td>
<td>Monterey Bay</td>
<td>Cold seep 34-67N, 122-19.92W</td>
<td>T</td>
<td>2,200</td>
<td></td>
</tr>
</tbody>
</table>

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* 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 Construction of the host mtDNA phylogenies was performed using NJ analyses of *C. okutanii* mtDNA sequences from the other, more distantly related taxa

* c christian wikimedia commons

* 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).
Table 2
Topoogy Tests Based on Vesicomyid Host and Symbiont DNA Sequence Data

| Comparison | Model | Thin | \( \rho_i \) | \( \hat{d}_{\text{max}} \) | AICM | MCSE | \( w_i \) | Log([f(X|Mi)]) | Log(BF) | Odds Ratio | P | 2log(BF) |
|------------|-------|------|-------------|-------------|------|------|--------|---------------|--------|------------|---|---------|
| w/ sym/clamBVII |        |      |             |             |      |      |        |               |        |            |   |         |
| COI versus mt16S | T2   | 2    | 0.01        | −3,380      | 66   | 6,825 | 3.6   | 0.99          | −3,393 |            |   |         |
| T1   | 2    | −0.03| −3,397      | 40          | 6,834 | 2.2   | 0.01  | −3,408        | 15     | 3.3 \( \times 10^6 \) | 0.00 | 30       |
| T2   | 2    | 0.03 | −5,837      | 76          | 11,750| 4.1   | 1.00  | −5,853        | 0.00   |            |   |         |
| T1   | 2    | −0.02| −5,899      | 50          | 11,868| 2.7   | 0.00  | −5,919        | 66     | 4.6 \( \times 10^8 \) | 0.00 | 132      |
| T2   | 2    | 0.02 | −6,658      | 70          | 13,385| 3.8   | 1.00  | −6,769        |        |            |   |         |
| 23S versus host | T2   | 2    | −0.003      | −6,761      | 51   | 13,572| 2.8   | 0.00          | −6,775 | 96          | 4.9 \( \times 10^4 \) | 0.00 | 192      |
| T1   | 2    | 0.04 | −5,429      | 64          | 10,922| 3.5   | 1.00  | −5,444        |        |            |   |         |
| T1   | 2    | 0.06 | −5,659      | 46          | 11,363| 2.5   | 0.00  | −5,668        | 224    | 1.9 \( \times 10^7 \) | 0.00 | 448      |
| T1   | 2    | 0.006| −5,362      | 69          | 10,792| 3.7   | 1.00  | −5,376        |        |            |   |         |
| soxA versus host | T2   | 2    | −0.003      | −5,547      | 49   | 11,143| 2.7   | 0.00          | −5,557 | 181         | 4.0 \( \times 10^7 \) | 0.00 | 362      |
| T1   | 2    | 0.03 | −3,361      | 57          | 6,779 | 3.8   | 1.00  | −3,374        |        |            |   |         |
| COI versus mt16S | T2   | 2    | −0.01       | −3,380      | 36   | 6,796 | 2.5   | 0.00          | −3,391 | 17          | 2.4 \( \times 10^7 \) | 0.00 | 34       |
| T1   | 2    | −0.01| −5,815      | 66          | 11,696| 3.6   | 1.00  | −5,832        |        |            |   |         |
| T2   | 2    | 0.01 | −5,842      | 46          | 11,730| 2.5   | 0.00  | −5,853        | 21     | 1.3 \( \times 10^9 \) | 0.00 | 42       |
| 23S versus host | T2   | 2    | 0.004       | −6,630      | 46   | 13,305| 2.5   | 0.00          | −6,638 | 18          | 6.6 \( \times 10^7 \) | 0.00 | 36       |
| T1   | 2    | 0.03 | −3,561      | 64          | 10,786| 3.5   | 1.00  | −3,577        |        |            |   |         |
| dnaK versus host | T2   | 2    | −0.01       | −5,407      | 47   | 10,861| 2.6   | 0.00          | −5,420 | 43          | 4.7 \( \times 10^8 \) | 0.00 | 86       |
| T1   | 2    | 0.01 | −5,320      | 62          | 10,702| 3.4   | 1.00  | −5,332        |        |            |   |         |
| T1   | 2    | 0.02 | −5,354      | 45          | 10,753| 2.5   | 0.00  | −5,367        | 35     | 1.6 \( \times 10^7 \) | 0.00 | 70       |
| soxA versus host | T2   | 2    | −0.003      | −13,034     | 74   | 26,143| 3.6   | 0.00          | −13,058| 61          | 3.1 \( \times 10^6 \) | 0.00 | 122      |
| T1   | 2    | 0.03 | −13,034     | 74          | 26,143| 3.6   | 0.00  | −13,058       |        |            |   |         |

w/o sym/clamBVII:

| Comparison | Model | Thin | \( \rho_i \) | \( \hat{d}_{\text{max}} \) | AICM | MCSE | \( w_i \) | Log([f(X|Mi)]) | Log(BF) | Odds Ratio | P | 2log(BF) |
|------------|-------|------|-------------|-------------|------|------|--------|---------------|--------|------------|---|---------|
| 16S versus host | T2   | 2    | 0.00        | −5,837      | 76   | 11,750| 4.1   | 1.00          | −5,853 | 0.00        |   |         |
| T1   | 2    | −0.02| −5,909      | 50          | 11,868| 2.7   | 0.00  | −5,919        | 66     | 4.6 \( \times 10^8 \) | 0.00 | 132      |
| T2   | 2    | 0.02 | −6,658      | 70          | 13,385| 3.8   | 1.00  | −6,679        |        |            |   |         |

16S from posterior simulation; Tary Material online).

(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 \( \times 10^6 \) 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, T1, in which both host and symbiont loci share the same topology, with a decoupling model, T2, 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 symBVII and its corresponding host clamBVII, as well as for data sets that lacked these sequences. The 6-locus analysis was run only for a data set lacking symBVII and clamBVII. If the data lent greater statistical support to T2, then a decoupling of symbiont and host mitochondrial phylogenies was indicated.

We used MrBayes to compare the marginal likelihood of the data under models T1 and T2. 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 T1
and T2, 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 \(5.0 \times 10^6\) 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 T1 and T2 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

\[
AICM = 2\hat{\ell}_{\text{max}} - 2\hat{d},
\]

where \(\hat{\ell}_{\text{max}}\) 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 \(\hat{\ell}_{\text{max}} = \bar{\ell} + \hat{s}_\ell^2\) and \(\hat{d} = 2\hat{s}_\ell^2\), where \(\bar{\ell}\) and \(\hat{s}_\ell^2\) are the sample mean and variance of the log-likelihood scores, respectively (Raftery et al. 2007). Therefore, AICM is

\[
AICM = 2(\bar{\ell} - \hat{s}_\ell^2).
\]

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

\[
\text{MCSE}_{AICM} = \sqrt{\frac{2\hat{d}}{B} + \frac{4\hat{d}}{B}\left(\frac{11\hat{d}}{4} + 12\right)}.
\]

(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, \(AICM_{\text{max}}\). The difference between the best model and other models that we wish to compare is \(\Delta AICM_i = AICM_{\text{max}} - AICM_i\), so that the Akaike weights for model \(i\) are defined as

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

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 (symBVII) that was highly divergent from that previously described for this host lineage (symAVII). This discovery prompted the hypothesis that symBVII 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 symBVII and applied statistical tests that confirm symbiont–host decoupling.

**Host Clam Phylogeny**

Sequence 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 symBVII (see below), was identical across both mitochondrial loci to a clam species previously identified as *Calypthena 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 clamAVII and that of the third individual as clamBVII. Bayesian analysis of the mitochondrial data (COI and mt16S concatenated) confirmed that clamAVII and clamBVII clustered in a monophyletic clade with the clam lineages *Vesicomya* sp. mt-I and -III from the eastern Pacific (PP = 0.97; fig. 1A).
The relationship of nucITS sequences from clamAVII and clamBVII 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. ClamAVII and clamBVII 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 clamAVII 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 symAVII. In contrast, the 16S of the individual bearing haplotype clamBVII was highly divergent, differing from symAVII at 14 sites (1.1\%; supplementary table 5, Supplementary Material online); we designate this divergent symbiont phylotype symBVII. The level of 16S divergence of symBVII 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 symBVII 16S sequence returned a top hit not to the *Vesicomya* sp. mt-II symbiont (symAVII) 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 symBVII fell in the monophyletic clade of vesicomyid symbionts with PP = 1.00 (fig. 2). Within this clade, the placement of the symBVII 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 symBVII 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 symBVII 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 symBVII and the *Vesicomya* sp. MAR symbiont, with PPs of 1.00 and 0.97, respectively (fig. 3). Nucleotide identities between symBVII 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 symBVII led us to examine the frequency at which this phylotype occurs in other individuals.
To do so, we sequenced the symbiont 16S from 115 additional clams collected from the JdF Ridge in 1995 and 1999 (Table 1). The symBⅢ 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 symBⅢ phylotype originally detected in the 1991 clam. Similarly, the host loci (COI, mt16S, nucITS) from this individual were identical to those of clamBⅢ (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 symAⅧ, the phylotype previously characterized for the Vesicomya sp. mt-II host lineage. The frequency of symBⅢ-hosting clams in the sampled populations was therefore 2 in 118 (0.02).

Upon first finding symBⅢ 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 symBⅢ in a second clam from a distinct population (JdF 1999), as well as by our multilocus characterization that unambiguously placed symBⅢ as sister to another vesicomyid symbiont.

Test for Symbiont–Host Coupling

The phylogenetic placement of symBⅢ with the symbiont of a distantly related host clam (Vesicomya sp. MAR)
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
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 symBVII provided the only information in the data rejecting symbiont–host coupling, we repeated the analysis excluding clamBVII and symBVII. Even without these sequences, the decoupling model T2 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 clamBVII and symBVII (table 3). The AU tests also provided evidence of decoupling, though congruence was reciprocally rejected for only 2 of the 4 symbiont loci (table 3).

Table 3

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff-lnL</th>
<th>AU</th>
<th>BIC</th>
</tr>
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<tr>
<td>w/ sym/clamBVII</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>COI</td>
<td>36.2</td>
<td>0.001</td>
<td>2 × 10⁻³⁶</td>
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<tr>
<td>mt16S</td>
<td>4.2</td>
<td>0.243</td>
<td>0.014</td>
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<td>COI + mt16S</td>
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<td></td>
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<tr>
<td>h5S</td>
<td>151.5</td>
<td>3 × 10⁻³²</td>
<td>2 × 10⁻⁷⁶</td>
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<tr>
<td>16S</td>
<td>47.7</td>
<td>0.003</td>
<td>2 × 10⁻²¹</td>
</tr>
<tr>
<td>23S</td>
<td>176.6</td>
<td>6 × 10⁻⁶⁸</td>
<td>2 × 10⁻²⁹</td>
</tr>
<tr>
<td>dnaK</td>
<td>218.9</td>
<td>8 × 10⁻⁵⁷</td>
<td>8 × 10⁻⁹⁶</td>
</tr>
<tr>
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</tr>
<tr>
<td>w/o sym/clamBVII</td>
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</tr>
<tr>
<td>COI</td>
<td>15.3</td>
<td>0.047</td>
<td>2 × 10⁻⁸⁷</td>
</tr>
<tr>
<td>mt16S</td>
<td>4.2</td>
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<td>0.011</td>
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<td>COI + mt16S</td>
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</tr>
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<td>h5S</td>
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<td>0.257</td>
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<td>0.084</td>
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</tr>
<tr>
<td>soxA</td>
<td>59.1</td>
<td>4 × 10⁻⁸⁶</td>
<td>4 × 10⁻⁸⁶</td>
</tr>
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<td>symBVII</td>
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<td></td>
</tr>
</tbody>
</table>

* First column for each test is for locus 1 data constrained by the ML topology for locus 2 data (Ho = 2); second column for each test is for locus 2 data constrained by the ML topology for locus 1 (Ho = 1); bold indicates instances of reciprocal rejection of topological incongruence (P < 0.05).

AIC: 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.

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

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, symBVII and the Vesicomya sp. MAR symbiont, occurring in distantly related hosts. SymBVII co-occurs with the previously characterized symbiont phenotype, symAIII (Peek et al. 1998; Goffredi et al. 2003), in clams of the Vesicomya sp. mt-II host lineage from the JdF Ridge. However, symBVII is highly divergent from symAIII 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 symBVII 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. kakei 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 symBVII returns a top hit to the symbiont of C. phaseoliformis. It therefore seems likely that symBVII 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
to the available samples; broader taxonomic sampling may reveal the source clam or population from which symBVII originated. Nonetheless, the clustering of symBVII and MAR shown here represents a clear uncoupling of symbiont and host phylogenies, suggesting lateral transfer of symBVII 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 symBVII, 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 vesticomysids, 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 vesticomysid 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 symbions and host mitochondria. Rather, it appears that vesicomyid symbions, though predominantly vertically transmitted (Peek et al. 1998; Hurtado et al. 2003), also experience occasional instances of lateral symbiont acquisition, with symBVII 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 symBVII entered the Vesicomya sp. mt-II host lineage.

**Potential Mechanisms of Lateral Symbiont Acquisition**

**Host Hybridization**

SymBVII 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 symbiotics 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 clamAVII and clamBVII, suggesting that clamBVII 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

![Fig. 5.—Hypothetical mechanisms of lateral (nonvertical) symbiont acquisition in vesicomyid clam symbioses. (A) Host hybridization hypothesis. Symbions (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.](image-url)
Horizontal Transfer

SymBVII 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

SymBVII 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; 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). Environmental acquisition has been suggested in other environments from a free-living pool of symbionts (fig. 2005), cichlid fishes (Hey et al. 2004), subspecies of chimpanzees (Won and Hey 2005), and possibly hydrothermal vent limpets (Johnson et al. 2006). 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 (Peak, Vrijenhoek, and Gaut 1998). Recent genome sequencing of 2 vesicomyid symbionts suggests that the symbiont genome (~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: ~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 symBVII 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 symBVII in Vesicomya sp. mt-II most likely resulted from a single lateral transfer event, followed by maintenance of symBVII at low frequency via vertical transmission. This is supported by the fact that the two clams hosting symBVII have exactly the same mitochondrial and symbiont haplotypes, suggesting a tight coupling of symbiont and mitochondrial genomes. Nonetheless, we cannot exclude the possibility that symBVII 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 Tigrionus 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 lineages of genus 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 symbionts. 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/).

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