

Genetic Subdivision of Chemosynthetic Endosymbionts of *Solemya velum* along the Southern New England Coast^{∇†}

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Population-level genetic diversity in the obligate symbiosis between the bivalve *Solemya velum* and its thioautotrophic bacterial endosymbiont was examined. Distinct populations along the New England coast shared a single mitochondrial genotype but were fixed for unique symbiont genotypes, indicating high levels of symbiont genetic structuring and potential symbiont-host decoupling.

Studies of endosymbioses between marine invertebrates and sulfur-oxidizing chemosynthetic bacteria have yielded tremendous insight into the biology of bacterium-eukaryote interactions. Though best described for deep-sea vents and cold seeps, these mutualisms, in which symbiont thioautotrophy supports the nutrition of both partners, are also ubiquitous in coastal sediments (17). Our understanding of these interactions stems largely from studies of symbioses involving protobranch bivalves in the family Solemyidae (16). Though solemyids and other species that form chemosynthetic symbioses occur globally, little is known about how symbionts and hosts are structured genetically across distinct populations. Characterizing these patterns is critical for understanding how symbiosis drives the coevolution of interacting species, as well as how environmental heterogeneity and dispersal affect local adaptation. This study examines the geographic structure of genetic variation in the symbiosis between chemosynthetic bacteria and the Atlantic protobranch *Solemya velum*.

Solemya velum is ideal for studying the evolution of highly coadapted bacterium-eukaryote mutualisms. This small bivalve (~1.5 to 3 cm) burrows in sulfide-rich coastal sediments, where it obtains most of its nutrition from thioautotrophic bacteria living within specialized gill cells (1, 10). Though observed from Florida to Canada (20), the distribution of *S. velum* is highly patchy, with seemingly suitable habitat often devoid of individuals (12). Consequently, molecular characterizations of this symbiosis have focused primarily on stable and locally abundant populations near Woods Hole, MA. Direct sequencing of the symbiont 16S rRNA gene from these individuals has revealed a single, unique phylotype clustering within the *Gammaproteobacteria* (5, 6, 9). DNA from this symbiont has been

extracted from *S. velum* ovarian tissue, raising the hypothesis that symbionts are transmitted vertically from mother to offspring (11) and are therefore tightly coupled to the host's life cycle and evolutionary history.

If symbiont acquisition is strictly vertical in *Solemya* populations, the genealogies of the symbiont and the cotransmitted host mitochondrion should diverge in parallel (cospeciation) (8, 15, 18). However, lateral acquisition involving either symbiont uptake from the environment or horizontal transfer between co-occurring hosts has not been ruled out for *Solemya* populations and could decouple symbiont and host genealogies (18). Indeed, 16S phylogenies show that symbionts of diverse *Solemya* species are polyphyletic, a pattern inconsistent with the putative monophyly of the hosts (based on nonmolecular characters) and suggestive of multiple evolutionary origins (2, 9, 16). However, tests for symbiont-host codiversification below the species level in *S. velum* are lacking; sequence data from multiple populations will help resolve questions of cospeciation and symbiont transmission in this group.

Here, distinct *Solemya velum* populations were genotyped to examine how symbiont diversity covaries with host diversity and geography. Individual bivalves ($n = 12$ to 22 per site) were collected from mudflats at four sites along the southern New England coast (Fig. 1A). DNA was extracted from the symbiont-containing gills and used for PCR amplification of fragments of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) and the symbiont 16S gene and hypervariable internal transcribed spacer (16S-ITS) (Table 1; also see the supplemental material). Unambiguous contigs of 340 nucleotides (nt) for the COI locus and 716 nt for the 16S-ITS locus, including 241 nt of the 16S and 475 nt (~95%) of the ITS, were generated via bidirectional direct sequencing of amplicons using BigDye chemistry. Symbiont identity was confirmed by blasting the 16S-ITS (Woods Hole [WH] phylotype) against an assembly of the *S. velum* symbiont genome from the same population (C. Cavanaugh, unpublished data). Blastn returned a single full-length hit with 100% identity across the locus. Genotype networks were then inferred via statistical parsimony in the program TCS (3).

Patterns of genetic diversity differed between host and symbiont in *Solemya velum* (Fig. 1B). Host COI sequences were largely homogenous across sampling sites, with a single genotype fixed across the Martha's Vineyard (MV), New Jersey (NJ), and WH populations. Individuals at the Rhode Island

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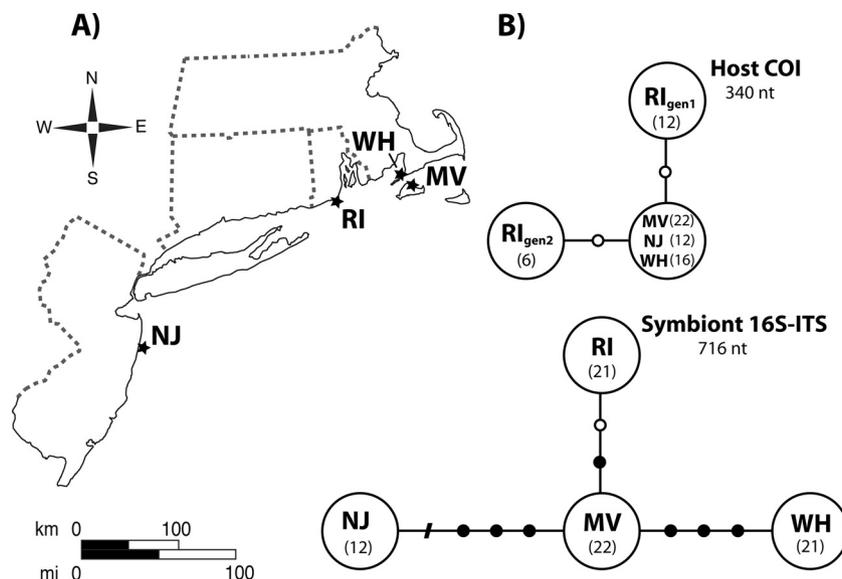


FIG. 1. (A) Locations of *Solemya velum* collection sites (stars) along the Atlantic Coast were Naushon Island, Woods Hole, MA (WH; 41.514°N, -70.712°W); Lake Tashmoo, Martha's Vineyard, MA (MV; 41.465°N, -70.623°W); Judith Pond, RI (RI; 41.380°N, -71.502°W); and Shark River Island, NJ (NJ; 40.186°N, -74.030°W). (B) Parsimony networks of host COI and symbiont 16S (241 nt) or symbiont 16S-ITS (716 nt) genotypes. Open circle, single-nucleotide substitution in either the host COI (top; 340 nt) or symbiont 16S (241 nt); filled circle, single-nucleotide substitution in the ITS portion (475 nt) of the 16S-ITS sequence fragment (716 nt total); diagonal bar, single-nucleotide indel in the symbiont ITS; gen1 and gen2, genotypes 1 and 2. Values in parentheses show the number of *S. velum* individuals from which sequences were obtained at each site.

(RI) site, situated between the NJ and WH-MV sites, exhibited two distinct genotypes at frequencies of 0.33 and 0.67, each differing from the MV-NJ-WH genotype by one single-nucleotide substitution (Fig. 1B). In contrast to the COI pattern, symbiont 16S-ITS variation was highly structured, with 100% of the diversity partitioned among sampling sites. Each site was characterized by one of four distinct 16S-ITS genotypes, each of which was fixed among all individuals from a site (mean pairwise F_{st} [23], 1.0). A total of nine polymorphisms (1.3% of the sequence) occurred across the four genotypes, with two to seven polymorphisms separating any two genotypes (Fig. 1B). These polymorphisms included one single-nucleotide indel and eight single-nucleotide substitutions, one of which occurred in the 16S gene 90 nt upstream of the ITS (Fig. 1B).

These data raise two primary hypotheses. First, *Solemya velum* symbiont populations are genetically subdivided. Despite the close proximity of sample locations (e.g., ~10 km separating WH and MV), no 16S-ITS genotypes were shared across sites. This partitioning differs from the pattern of ITS variation in other chemosynthetic symbionts. Notably, vertically transmitted symbionts of the vent clam *Calypotogena mag-*

nifica were shown to display identical ITS sequences across hosts separated by thousands of miles (8). Similarly, identical symbiont ITS genotypes were found in tubeworms (*Riftia pachyptila*) from vent sites at 18°S and 9°N on the East Pacific Rise and in the Gulf of California (27°N) (4), despite the fact that *R. pachyptila* acquires symbionts laterally, presumably from the bacterial community at the larval settlement site (7, 14). Our data suggest that mixing of *S. velum* symbionts across sites may be constrained relative to mechanisms imposing genetic structure, which potentially include physical barriers to symbiont dispersal or site-specific selection of locally adapted symbiont genotypes by hosts (as postulated for squid *Vibrio* symbionts [22]). Symbionts spanning the *S. velum* host range (Florida to Canada) may therefore exhibit substantial genetic variation, some of which may underlie adaptations to geographic differences in host physiology or environment (e.g., temperature or sulfur concentration).

Second, symbiont and host genetic variation are not definitively coupled in *Solemya velum*. In contrast to the symbiont data, host COI sequences imply higher connectivity among sites, with distinct locations (from MV to NJ) sharing identical

TABLE 1. Symbiont and host primers used in PCR^a and direct sequencing

Locus, source of DNA	Primer	Sequence (5' to 3')	Amplicon length (nt) ^b	Sequenced length (nt) ^c
16S-ITS, symbiont	16S 937F	ACGCGAAGAACCCTTACCAGCTCTT	~1,100 ^d	716
	23S 37R	AACGTCCTTCATCGCCTCTTACCG		
COI, host	COI 2F	TGAGCCGGTATAGTTGGAACATC	500	340
	COI 546R	ATTGCTCCGGCTAGAACTGGAAGT		

^a PCR parameters were 2 min at 92°C; 30 cycles of 25 s at 92°C, 25 s at 50°C, and 90 s at 72°C; and 5 min at 72°C using Herculase polymerase (Stratagene).

^b Length of amplified PCR product.

^c Length of unambiguous bidirectional sequence recovered per individual.

^d 16S-ITS primers span 551 nt of the 16S gene (3' end), the ITS (~500 bp), and 37 nt of the 23S gene (5' end).

genotypes. The RI population is an exception to this pattern, suggesting that the RI site, an estuary linked to the ocean by a narrow inlet, may be isolated from processes connecting the MV-NJ-WH sites. The discrepancy between the symbiont and host data could be explained by substitution rate variation between loci, with the COI locus unable to resolve subdivisions apparent in the 16S-ITS data; sequencing of more rapidly evolving host loci may reveal genetic structure consistent with that of the symbiont marker. Alternatively, symbiont and host lineages may be physically decoupled, perhaps due to lateral symbiont acquisition by the hosts. The data are indeed consistent with the hypothesis that dispersing hosts acquire their symbionts from geographically structured free-living bacterial populations. Alternatively, free-living bacteria may be mixed across sites, with geographic structure among the endosymbiont populations imposed by hosts selecting locally adapted genotypes from the environmental pool. These hypotheses warrant rigorous testing, as determining the mode of symbiont acquisition is critical for understanding processes of symbiont genome evolution (e.g., recombination or genome reduction) (13, 19, 21). Our data suggest the need to reevaluate transmission dynamics in *Solemya velum* and highlight this symbiosis as a potential model for phylogeographic studies of coevolving species.

Nucleotide sequence accession numbers. The sequences determined in this study have been deposited in the GenBank database with accession numbers GQ280812 to GQ280820.

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