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Archaea are ubiquitous in marine plankton, and fossil forms of archaeal tetraether membrane lipids in sedimentary rocks document their participation in marine biogeochemical cycles for >100 million years. Ribosomal RNA surveys have identified four major clades of planktonic archaea but, to date, tetraether lipids have been characterized in only one, the Marine Group I Thaumarchaeota. The membrane lipid composition of the other planktonic archaeal groups—all uncultured Euryarchaeota—is currently unknown. Using integrated nucleic acid and lipid analyses, we found that Marine Group II Euryarchaeota (MG-II) contributed significantly to the tetraether lipid pool in the North Pacific Subtropical Gyre at shallow to intermediate depths. Our data strongly suggested that MG-II also synthesizes crenarchaeol, a tetraether lipid previously considered to be a unique biomarker for Thaumarchaeota. Metagenomic datasets spanning 5 y indicated that depth stratification of planktonic archaeal groups was a stable feature in the North Pacific Subtropical Gyre. The consistent prevalence of MG-II at depths where the bulk of exported organic matter originates, together with their ubiquitous distribution over diverse oceanic provinces, suggests that this clade is a significant source of tetraether lipids to marine sediments. Our results are relevant to archaeal lipid biomarker applications in the modern oceans and the interpretation of these compounds in the geologic record.

Significance

All three domains of life—Eukarya, Bacteria, and Archaea—are known to inhabit the marine water column from surface waters to great depth. Planktonic marine Archaea are comprised of two dominant groups—the Thaumarchaeota and Euryarchaeota. The Thaumarchaeota contain characteristic biomarker lipids known as tetraethers that are thought to be diagnostic for this group, and are used as paleotemperature proxies, since these lipids are well preserved in marine sediments. In this study, we show that planktonic Euryarchaeota produce the same types of archaeal tetraether lipids as do Thaumarchaeota. Our results have important implications for environmental surveys of marine Archaea, and the use of their lipids for interpretation of the sedimentary record.

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The authors declare no conflict of interest.

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Data deposition: The sequence reported in this paper has been deposited in the NCBI sequence read archive, www.ncbi.nlm.nih.gov/sra (accession no. SRP042245, SRX553024, SRX553023, SRX556067, and SRX556088).

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The 16S rRNA amplicon data provided a more detailed taxonomic breakdown of MG-I and MG-II phylotypes in the different size fractions and depths. We focused on samples from eight depths that yielded >1,000 amplicons in each size fraction (SI Appendix, Figs. S5 and S6 and Table S2), and the 0.22- to 1.6-μm size fraction from 800 m and 1000 m. In total, this sample set contained 323,924 archaeal amplicon sequences after curation and quality control. Archaeal rRNA sequences were clustered into operational taxonomic units (OTUs) using the software program QIIME (41) and taxonomy assigned through comparison with the ARB-SILVA SSU reference database using BLASTn.

The 10 most prevalent archaeal OTUs across the entire dataset (each representing >2,000 amplicon sequences and, in total, 82% of archaeal amplicons) were assigned to either the Thaumarchaeota (primarily MG-I) or MG-II Euryarchaeota (Fig. 1). Among the Thaumarchaeota, OTU D was predominant in both size fractions of SPM and represented a higher percent of total archaeal OTUs with increasing depth. At 1,000 m, OTU A had a higher relative abundance than OTU D, and OTU C rose in abundance, consistent with previous reports of genetic diversity of Thaumarchaeota in depth profiles (22).

In sharp contrast to Thaumarchaeota, MG-II Euryarchaeota represented a much higher proportion of total archaeal OTUs at shallower depths, and the MG-II population was not dominated by a single phylotype (Fig. 1). Four OTUs—E, F, H, and I—were abundant in the 131- and 157-m samples, and OTU I became more prominent with depth. Such intragroup stratification may reflect niche partitioning, since some MG-II photic zone eukaryote types encode the light-driven proton pump proteorhodopsin (42, 43), whereas mesopelagic MG-II eukaryote types tend to lack the proteorhodopsin gene (42). Patterns of MG-II OTU abundance also varied between size classes; at 131 m, for instance, OTU F was more highly represented in the 3- to 57-μm fraction than in the 0.3- to 3-μm fraction.

The near-binary nature of the archaeal community in the sample set enabled us to estimate MG-II cell densities using MG-I rRNA qPCR data and the relative abundances of MG-I and MG-II phylotypes in the different size fractions. The deep North Atlantic (35), and the East China Sea (36). The potential contribution of this cosmopolitan group to the marine tetrathiaer lip pool has been debated (37–40), but the lack of cultivated representatives of MG-II has precluded direct analysis of their membrane lipids, and incomplete knowledge of the genetic basis of archaeal tetrathiaer lipid biosynthesis limits the ability of metagenomic studies to address this question.

We conducted parallel characterization of planktonic archaeal lipids and archaeal community DNA composition to constrain the taxonomic origins of GDGTs in marine plankton and their distribution in the marine water column. Metagenomic analyses, quantitative polymerase chain reaction (qPCR) of Thaumarchaeota marker genes, and small subunit ribosomal RNA (SSU rRNA) gene amplicon pyrosequencing were used to characterize the overall archaeal community composition in size-fractionated suspended particulate matter (SPM). Parallel measurements of archaeal tetrathiaer lipids from the same filter samples enabled us to associate GDGTs with particular members of the planktonic archaeal community.

### Results and Discussion

SPM for this study was collected in the North Pacific Subtropical Gyre (NPSG) in September 2011 using in situ pumps deployed at depths ranging from 11 to 559 m (SI Appendix, Figs. S2 and S3). SPM was fractionated into two size classes (0.3–3 μm and 3–57 μm) in an effort to separate the archaeal community into free-living and particle-associated/detrital components. Lipids and DNA (SI Appendix, Fig. S4) were extracted from the same filters.

#### Depth Distributions of Archaeal Phyla

We characterized archaeal communities in our sample set using two independent techniques: metagenomics and pyrosequencing of amplicons of the V1–V3 region of the SSU rRNA gene in DNA. Counts of euryarchaeotal and thaumarchaeotal 16S rRNAs and functional gene sequences in metagenomic datasets (Table 1) indicated that Euryarchaeota outnumbered Thaumarchaeota at depths above 157 m in the 0.3- to 3-μm size fraction and in the 3- to 57-μm size fraction throughout the entire profile. Notably, at 83 m depth, the archaeal population was comprised nearly exclusively of MG-II.

#### Table 1. Archaeal community composition and relative abundances of crenarchaeol and GDGTs with zero to three cyclopentyl moieties in suspended particulate matter samples

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Size fraction</th>
<th>% Eury</th>
<th>Relative abundance of GDGT cores</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3–3</td>
<td>83</td>
<td>97.2</td>
<td>22.4</td>
</tr>
<tr>
<td>131</td>
<td>74.4</td>
<td>66.7</td>
<td>21.8</td>
</tr>
<tr>
<td>3–57</td>
<td>100.0</td>
<td>96.1</td>
<td>36.6</td>
</tr>
</tbody>
</table>

*Hydrolysates of total lipid extracts.

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and MG-II determined from metagenomes (Eq. 1 and Fig. 2 A and B). Overall, MG-I and MG-II distributions were similar to those reported in a previous NPSG study (22). Two independent metrics we report here also support the dominance of MG-II in the upper ocean: At 83 m, MG-I were not detectable by qPCR (Fig. 2 A and B), and Euryarchaeota comprised >94% of the 16S rRNA and archaeal metagenomic read annotations (MGAs) (Table 1).

Both MG-I and MG-II DNAs were more abundant in the 0.3- to 3-μm than in the 3- to 57-μm size fraction, consistent with a predominantly free-living existence (FISH) (21, 24). Both size class MG-I profiles (Fig. 2 A and B) showed a gradual increase in cell density with depth, and suggest a small percentage (~10–15%) of a predominantly free-living thaumarchaeotal population was captured on the large filters.

MG-II depth profiles showed more pronounced differences between size fractions, with MG-II cells captured on the large filter ranging from 2% to 90% of the total MG-II detected at a given depth. This variability may be related to differences in cell size and the extent of particle association between ecotypes, as MG-II have been reported to be pleomorphic and larger than MG-I cells (44).

**Tetraether Lipid Distributions and Partitioning.** Core GDGT molecules (SI Appendix, Fig. S1) are composed of two isoprenoidal 
C₄₀ hydrocarbon cores linked to glycerol by ether bonds at each end. Many GDGTs contain internal cyclopentyl moietyes, and crenarchaeol has an additional six-membered ring (SI Appendix, Fig. S1). In intact polar lipid (IPL) GDGTs, glycerol is linked to polar head groups, often hexose moieties. Because polar head groups have been found to degrade rapidly after cell death (45), IPLs in sediments have been proposed as indicators of live biomass. It has been presumed that only a small proportion of GDGTs in live cells exist as free core GDGTs, but how the relative proportion of these lipids varies with environmental conditions, taxa, or growth stages, is not well constrained.

We measured core GDGTs in lipid extracts using high-performance liquid chromatography–atmospheric pressure chemical ionization (APCI) mass spectrometry (46, 47). In the small size fraction samples, free core GDGT concentrations were 1–20 pg/L and peaked at 157 m. Free core GDGTs (Fig. 2 C and D) were most abundant in the large size fraction, where their concentrations ranged from 16 to 490 pg/L. They reached an upper water column maximum at 331 m, but the highest concentration was measured at 559 m. Similar partitioning, with a higher proportion of core GDGTs detected in the larger size fraction, was reported in a study of size-fractionated SPM collected in Puget Sound (48). Notably, concentrations of core GDGTs (including crenarchaeol) in the 83-m samples dominated by MG-II (94–100% Euryarchaeota) were similar to those in deeper (>300 m) samples that had high MG-I representation (Fig. 2 C and D).

**Intact Polar GDGTs.** Several series of very late eluting peaks were prominent in extracted ion chromatograms of masses corresponding to GDGTs 0–3 and crenarchaeol in the small size fraction samples from 131 and 559 m (Fig. 3 and SI Appendix, Fig. S7). Within each series, the order of retention times was identical to that of commonly detected free GDGTs. Because they eluted at the stage of analysis at which HPLC eluents are most polar, we postulated that these peaks represented IPL GDGTs that lost their head groups during in-source fragmentation in the mass spectrometer. A hydrolysis experiment supported the IPL GDGT hypothesis: After the extracts were treated with hydrochloric acid (49), the late-eluting peaks disappeared and the peak areas of core GDGTs increased (Fig. 3 and SI Appendix, Fig. S7). This difference was most pronounced in the 131-m sample, in which concentrations of IPL-bound GDGTs were 123 pg/L, exceeding those of free core GDGTs (5 pg/L) by more than an order of magnitude. Euryarchaeotal DNAs were predominant in this sample (74% euryarchaeotal DNAs, 26% thaumarchaotal). At 559 m, the depth at which Thaumarchaeota reached a maximum, IPL-bound GDGT concentrations were

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**Fig. 2.** Inferred cell densities of Thaumarchaeota and Euryarchaeota and core GDGT lipid concentrations in small (A and C) and large (B and D) size fractions of suspended particulate matter in the NPSG. MG-I cell densities (solid blue line) were determined by qPCR, and were below limits of detection at depths <100 m. MG-II cell densities (dashed red line) were inferred from archaeal community composition as determined from MG-I cell densities using SSU rRNA gene representation in metagenomic datasets (Eq. 1). Mean values of replicate GDGT analyses are shown; error bars indicating the range of measurements are smaller than the data points. Note variable scales on x axes.

**Fig. 3.** HPLC-APCI-MS composite extracted ion chromatograms (EIC) of total lipid extract (TLE) from 131 m, 0.3–3 μm size fraction sample before (A) and after (B) acid hydrolysis. Colored traces are EICs of m/z values of individual core GDGTs and the hydrolysis-resistant internal standard. Late eluting peaks in A represent three putative series of core GDGTs released from IPL GDGTs by fragmentation in the APCI source. After acid hydrolysis, late-eluting peaks disappeared and the peak area of core GDGTs increased, supporting this interpretation.
Marine Euryarchaeota Contain Crenarchaeol and Other Tetraether Lipids. Two lines of convergent evidence indicate that MG-II Euryarchaeota are a major source of tetraether lipids in the NPSG and, by extrapolation, throughout the open ocean and in coastal waters where Euryarchaeota are found (3, 23–25). First, core GDGTs—including crenarchaeol—were detected in both SPM size fractions throughout the profile, most notably at depths at which MG-II dominated the archaeal population (Fig. 2). The 83-m samples composed nearly exclusively of Euryarchaeota (95–100%) contained as much core GDGTs as deeper samples with higher MG-I representation (Fig. 2 C and D). Because the turnover time of cells in the upper water column is on the order of a few days (52), the free core GDGTs we measured must have been derived from living or recently living archaea found at those depths. IPL-bound GDGTs (53–55) provide a second line of evidence for a MG-II contribution to the tetraether lipid pool. Concentrations of these lipids at 559 m (dominated by MG-I) were less than one-fifth those measured at 131 m (dominated by MG-II). It therefore appears unlikely that MG-I, less abundant at 131 m than at 559 m, could be the sole source of the much higher IPL GDGT concentrations detected in the shallower sample.

Similar distributions of MG-I and MG-II were reported in a previous metagenomic study using samples collected in 2002–2004 (22); this continuity suggests that the pattern of MG-II dominance in the upper open ocean is not ephemeral. To improve the temporal resolution of the historical record, we queried datasets from four metagenomic profiles and one metatranscriptomic profile generated from the NPSG against a comprehensive database that included a draft MG-II metagenome (43). Subsequent analysis of the relative contributions of Euryarchaeota and Thaumarchaeota to total protein-coding reads (SI Appendix, Fig. S9) revealed a pattern similar to that seen in the amplicon dataset. Thus, fosmid, amplicon, and pyrosequenced metagenomic datasets from samples spanning nearly a decade all indicated that euryarchaeotal dominance in the upper ocean is a persistent feature of the NPSG.

Our finding that Euryarchaeota are a major source of GDGTs in the water column is consistent with the observation that tetraether lipid biosynthesis—a broadly but not universally distributed trait among Euryarchaeota—is common among members of the Thermoplasmatales (56), the most closely related cultivars to MG-II. Moreover, biosynthesis of crenarchaeol by MG-II was previously proposed on the basis of water column GDGT and DNA distributions (37–40). A role for the cyclohexyl moiety in maintaining tetraether membrane fluidity at low temperatures, possibly enabling the expansion of thermophilic MG-I from hydrothermal environments to the open ocean, was postulated (17) but later contradicted by reports of crenarchaeol in hot springs (57, 58) and thermophilic MG-I isolates (18, 59).

Although crenarchaeol biosynthesis has been confirmed in MG-I isolates (e.g., ref. 16), determining its sources—and sources of other tetraether lipids—in environments in which multiple archaeal groups coexist has been difficult. Studies examining correlations between crenarchaeol and MG-I SSU rRNA or amoA gene transcript copy numbers in the water column may have been complicated by an early focus on core rather than IPL GDGTs, and the use of filters with different pore sizes for collection of lipids and DNA, and for FISH counts. The absence of an apparent correlation between community composition and the relative proportions of individual GDGTs detected in this study (Table 1) suggests that it may not be possible to structurally differentiate marine planktonic GDGT pools in the case of mixed MG-I and MG-II archaeal populations.

Geological and Ecological Implications. Together with the widespread predominance of MG-II in the epipelagic zone, where exported GDGT signals have been reported to originate (60, 61), our evidence that MG-II are a source of tetraether lipids suggests that a significant proportion of GDGTs delivered to sediments are likely synthesized by Euryarchaeota. This finding has important implications for past and future oceanographic and organic geochemical studies, and may necessitate a reinterpretation of archaeal tetraether lipid distributions in the geologic record.

The paleotemperature proxy TEX86 (TetraEther indiX of tetraethers containing 86 carbon atoms) relies on an empirical relationship between sea surface temperature (SST) and relative abundances of GDGTs in sediments that have been presumed to be derived from planktonic Thaumarchaeota (9, 62). Although TEX86 is now widely used in paleoceanography and core top calibration studies have found correlations between temperatures inferred using it and SSTs to be strong (62), mechanisms underlying the proxy are not well understood (63). One critical but as yet unresolved issue is how SST signals can be closely reflected in the membrane lipid composition of planktonic Thaumarchaeota, which are typically less abundant in epipelagic waters, where much exported organic matter originates. Tetraether lipid biosynthesis by MG-II provides a possible solution: If temperature affects the membrane lipid composition of MG-II Euryarchaeota, MG-II GDGTs are more likely than those of deeper-dwelling MG-I Thaumarchaeota to reflect sea surface temperatures. Significant physiological differences between the groups, however, may complicate this scenario. Diverse heterotrophic MG-II populations may be influenced by very different ecological factors than those that impact chemoolithoautotrophic MG-I populations. Heterotrophic MG-II populations likely respond to changes in organic matter availability and primary productivity, and their abundances have been observed to vary seasonally in a variety of habitats (24, 64).

Because TEX86 relies on relative rather than absolute abundances of GDGTs to estimate SST, it may in theory be immune to changes in GDGT flux or changes in community composition, provided that temperature is the only factor influencing archaeal membrane lipid composition in the ocean. This assumption is probably too simplistic, because the tetraether lipid composition of cultured archaea is known to vary with factors such as pH (65).

Analyses of one sample in this study (440 m, small size fraction) underscored the fact that our understanding of factors influencing relative proportions of GDGTs in the water column remains incomplete. This deep sample showed a GDGT pattern atypical of marine waters (SI Appendix, Fig. S10), with GDGT-2 more abundant than GDGT-0 or crenarchaeol—yet the archaeal community was dominated by the same MG-I OTU as at other depths with similar in situ temperatures. Neither temperature nor community composition seem likely explanations for this unusual GDGT signal, although the full genetic diversity of MG-I ecotypes is not entirely reflected in RNA data.

A mixed biological origin for GDGTs detected in marine sediments may confound efforts to use universally applicable paleoceanographic tools. A recent attempt to detect marine carbon isotope excursions by measuring the carbon isotopic composition (δ13C) of GDGTs (66) made the assumption that these compounds were synthesized by autotrophic Thaumarchaeota and argued that the δ13C of GDGTs must covary with that of the dissolved inorganic carbon (DIC) substrate. If, however, heterotrophic Euryarchaeota also contributed to the GDGT pool (as now appears likely), the isotopic signal would be mixed,
reflecting the $^{13}$C of their organic carbon substrates as well as that of DIC.

Conclusions

The results presented should help guide future studies of planktonic archaea and their lipids in the marine water column and sediments. The enrichment and isolation of representative strains of MG-II would greatly aid attempts to understand its ecology, physiology, and membrane lipid composition, but further cultivation-independent studies are also likely to yield new information about this important group of marine plankton.

Isotopically labeled tracer experiments have high potential for probing MG-II populations. Early microcosm studies using $^{13}$C labeled bicarbonate amendments (67) were valuable in predicting the physiology of MG-I communities before any representativeness were isolated. More recently, $^{13}$C tracer experiments have been successful in attributing the production of specific IPL classes to their autotrophic/heterotrophic bacterial sources (68). Similar experiments using labeled organic substrates may prove equally informative regarding the physiology and membrane lipid composition of MG-II Euryarchaeota.

In addition to better-constrained GDGT sources, a more complete knowledge of export processes and residence times will be necessary to interrelate the distributions of GDGT-containing archaea in the water column with their lipid profiles in the sedimentary record. Sediment trap studies, coupled with lipid and nucleic acid analyses of SPM and sediments, may reveal the depths from which exported GDGTs originate. Such investigations, conducted in both open ocean and coastal environments, in transects, profiles, and time series, should also help constrain the balance of euryarchaeotal/thaumarchaeotal lipids exported to the seafloor in different oceanic regions and seasons. Further analyses of size-fractionated SPM, combining archaeal community and lipid composition measurements with compound-specific radiocarbon analyses and thorium-based measures of particle flux, may also yield more insight into the mechanisms and timescales of GDGT export.

Materials and Methods

Sample Collection. Size-fractionated SPM samples (0.3–3 μm and 3–57 μm) were collected in September 2011 (SI Appendix, Figs. S2 and S3; details in SI Appendix) near Hawaii Ocean Time-series Station ALOHA using in situ pumps fitted with glass fiber filters.

DNA Methods. DNA was extracted from filter sections corresponding to 20 L of seawater (SI Appendix, Fig. S4). The qPCR assays used primers MGI_751F; S-GTCATACAGAACAYTCT and MGI_956R; S-HGGCGTGATCCTAAATGG and generally followed the program of Mincer et al. (28) (details in SI Appendix). We amplified the V1–V3 variable region of the archaeal SSU rDNA gene using the primers 20F S-TCGGTTGATCCYGCCRG-3′ (23) and a barcoded reverse primer 519R, S-GGDTTATACGGCGCCTG-3′ (69). Pooled amplicons were sequenced using the 454 Genome Sequencer (Roche; details in SI Appendix), using AmpliconNoise (70) to remove 454 sequencing errors, and trimming reads with SortMeRNA (78) to identify those containing rRNA sequences (SI Appendix); taxonomy was determined by using BLASTn (top hit, e-values <0.0001, bit scores >50) to compare with a reference database comprised of combined SILVA release 106 SSU and LSU databases and a SS database (80). Using BLASTnX or LAST (79), non-rRNA reads were compared with a reference database (SI Appendix) and their taxonomic affiliation determined in MEGAN (81). The proportion of thaumarchaeal and euryarchaeal reads was calculated as a percent of total reads assigned at or below phylum level.

Lipid extraction, hydrolysis, and analysis. Lipids were extracted from glass fiber filters containing SPM following a modified Bligh–Dyer protocol (50) (details in SI Appendix). A C46 tetraether (82), which lacks a polar head group and is unmodified by acid hydrolysis, was used as an internal standard. Aliquots of TLE were subjected to acid hydrolysis to cleave head groups of intact polar lipids, which may also yield more insight into the mechanisms and timescales of GDGT export.

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32. Hugoni M, et al. (2013) Structure of the rare archaeal biosphere and seasonal dy-
38. Turich C, et al. (2007) Lipids of marine Archaea: Patterns and provenance in the water-