Assessing the Use of Archaeal Lipids as Marine Environmental Proxies

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Abstract

Archaea are abundant in marine and terrestrial aquatic environments, sediments, and soils. They inhabit at least an 85°C temperature range from the polar ocean to hydrothermal springs. Many Archaea produce membrane lipids called glycerol dialkyl glycerol tetraethers (GDGTs). Experiments on pure and enrichment cultures as well as an empirical correlation for marine sediments (the TEX_{86} index) together show positive relationships between temperature and the number of cyclopentane or cyclohexane rings in GDGTs. The resulting TEX_{86} paleotemperature proxy has been applied across a wide range of geologic history and depositional settings. The exact relationship between TEX_{86} and temperature, however, remains poorly understood. Environmental systems and cultures have different temperature dependencies, and the ecological niche(s) of aquatic Archaea are still a subject of active investigation. Here we review some of the remaining questions about GDGT paleotemperature proxies. Better answers to these challenging problems will help refine future paleoceanographic applications.

1. INTRODUCTION

Biphytanyl: having two phytane units (C_{40} isoprenoid)

Phytanyl: having one phytane unit (C_{20} isoprenoid)

GDGT: glycerol dialkyl glycerol tetraether

IPL: intact polar lipid

More than 30 years ago, biphytanyl (C_{40}) isoprenoid hydrocarbon chains of Archaea were discovered in sedimentary systems (e.g., Chappe et al. 1982, Michaelis & Albrecht 1979, Moldowan & Seifert 1979). Given the diagnostic central linkage of the two phytanyl groups and identification of the parent biomolecules in hyperthermophiles (Langworthy 1977), it was understood that Archaea must be the source of these compounds. Early explanations often invoked a methanogenic origin, despite the incompatibility of compound-specific $\delta^{13}C$ measurements with this interpretation (e.g., Kohnen et al. 1992) and the general preference of methanogens for diethers with C_{20} isoprenoid chains (e.g., archaeol) (Tornabene & Langworthy 1979). As more data accumulated, however, the appearance of a regular pattern of C_{40} isoprenoid hydrocarbons in marine sediments, water column–suspended particulate matter, and ancient sedimentary deposits pointed to a source that must be more environmentally widespread (Hoefs et al. 1997).

The discovery of mesophilic Archaea thus marked a turning point for organic geochemists. Early studies of marine prokaryotic diversity based on sequencing 16S ribosomal RNA genes revealed planktonic Archaea in both the Atlantic and Pacific Oceans. Two major clades—called Group I and Group II—initially were classified as members of the Crenarchaeota and Euryarchaeota, respectively (Delong 1992, Fuhrman et al. 1992). Recently, the Group I Archaea and their close relatives from terrestrial environments have been reclassified as a separate phylum. Called Thaumarchaeota, these organisms may be the most ancient Archaea, i.e., they are not descendants of the known thermophiles (Brochier-Armanet et al. 2008, Pester et al. 2011, Spang et al. 2010). Many examples now confirm that Thaumarchaeota are among the most abundant organisms in the ocean, approaching 40% of all cells in the bathypelagic (e.g., Karner et al. 2001), whereas the Group II Euryarchaeota, in general, are found in lower numbers and favor the surface ocean (Herndl et al. 2005, Pernthaler et al. 2002).

The lipids of these Archaea are commonly known as glycerol dialkyl glycerol tetraethers (GDGTs), short for *sn*-2,3-di-*O*-biphytanyl diglycerol tetraethers (**Figure 1**). The core structures are most often abbreviated as GDGT-0, GDGT-1, GDGT-2, GDGT-3, and GDGT-4, with the numbers denoting internal cyclopentane rings. The GDGT containing a cyclohexane group is called crenarchaeol and is abbreviated as cren or GDGT-5. A related compound that has a longer chromatographic retention time (**Figure 2**) is known as the crenarchaeol regioisomer (cren′, GDGT-5′) (Sinninghe Damsté et al. 2002, Schouten et al. 2000). Phospholipids and glycolipids are the most common intact polar lipid (IPL) forms of the original biomolecules.

Because these archaeal lipids have high preservation potential, over at least the past 100 million years (Kuypers et al. 2001, Schouten et al. 2003a), researchers quickly recognized that useful paleoclimatological proxies could be developed using the properties of these lipids. In aquatic systems, the majority of organic matter deposited to sediments is believed to be of planktonic origin, and accordingly, sinking and suspended particulate matter contains the expected suite of core GDGT compounds (Huguet et al. 2007; Ingalls et al. 2006; Turich et al. 2007; Wuchter et al. 2005, 2006b). Sediments deposited in oxic and anoxic environments, e.g., oceans, lakes, soils, and hot springs, all contain archaeal GDGTs (Hopmans et al. 2000, Schouten et al. 2000). This ubiquity gave new promise to historical research on the correlation between growth temperature and the number of cyclopentane rings: In hyperthermophiles, the number of rings had been observed to increase with growth temperature (Derosa et al. 1980, Gliozzi et al. 1983). As a result of these findings, a paleotemperature proxy was on the horizon with the potential to unlock clues to ocean temperatures as far back as the Cretaceous (Schouten et al. 2002).

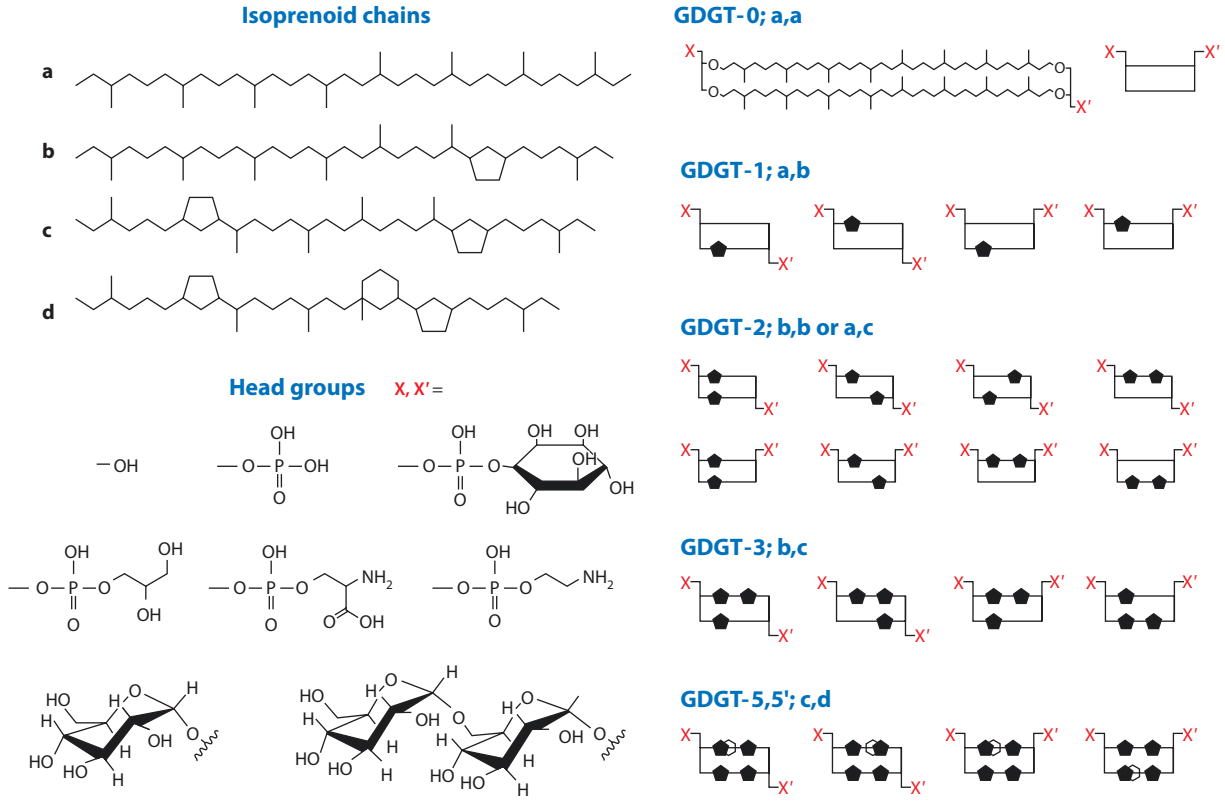

Figure 1

Glycerol dialkyl glycerol tetraether (GDGT) lipids of mesophilic Archaea. The C_{40} isoprenoid hydrocarbon backbones **a**, **b**, **c**, and **d** contain variable numbers of cyclopentane rings, plus, in the case of backbone **d**, one cyclohexane ring. Core GDGT lipids are composed of two of these hydrocarbon chains, linked at either end to the C_2 and C_3 carbon atoms of glycerol. The C_1 carbon atom of glycerol is connected to a polar head group, X. In core lipids, X is a hydroxyl group (-OH), whereas the intact polar lipid, or biological form, is one of many possible groups including—but not limited to—the phospholipid and glycolipid options shown here. The vast number of combinations of these components gives rise to many structural isomers, as symbolized on the right side of the figure. Here, the isoprenoid backbones are symbolized as lines containing the five- and six-membered rings of structures **a**, **b**, **c**, and **d**. There are only two possible arrangements for the GDGT-0 structure: each isoprenoid connected at one end to C_2 and at the other to C_3, versus one isoprenoid connected at both ends to C_2 and the other at both ends to C_3. The latter option is known as the "regioisomer" form, and it is believed to occur for all GDGTs. The total number of structural options increases when rings are added to the isoprenoids; if X and X′ are different, there are up to twice as many structural isomers as shown. However, in general, these many structures are not resolved during chromatographic analysis of GDGTs, i.e., all four options may underlie the peak called GDGT-3 in **Figure 2**.

2. THE PROPOSED PALEOTHERMOMETER

2.1. Physiological Basis for a Relationship Between Temperature and GDGTs

Many types of membrane adaptations, including cyclization as well other structural variations, help Archaea to occupy Earth's diverse range of habitats (Koga & Morii 2007). Although any combination of GDGTs can result in the required liquid crystal membrane state (from 0 to 100°C) (Ulrih et al. 2009), different species living in the same conditions can have vastly different membrane compositions. GDGTs confer stability to membranes (Brown et al. 2009, Chong 2010, Komatsu & Chong 1998), but the dominance of diether lipids in *Methanopyrus kandlerii* growing

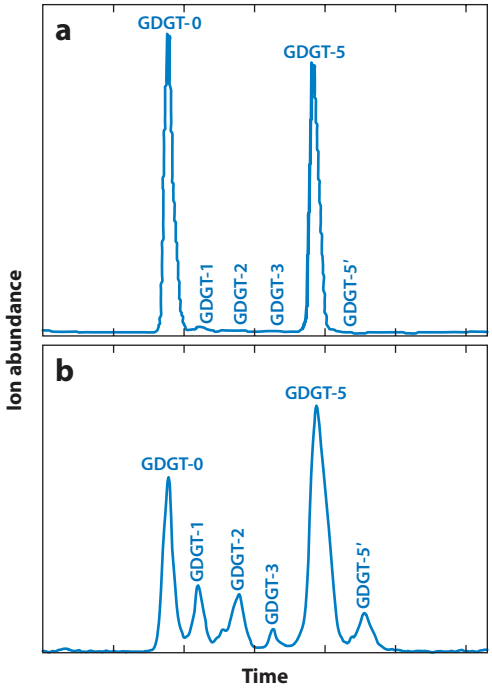

Figure 2

High-performance liquid chromatography–mass spectrometry is used to separate archaeal GDGTs. The area under each peak in the ion chromatogram is believed to reflect its abundance. Samples depicted are (*a*) typical for colder-water environments, with GDGT-0 ≥ crenarchaeol and low abundance of GDGT-1 to -3 and the crenarchaeol regioisomer, and (*b*) typical for warmer-water environments, with GDGT-0 < crenarchaeol and greater abundance of GDGT-1 to -3 and the crenarchaeol regioisomer (A. Pearson and A.E. Ingalls, unpublished chromatograms). Abbreviations: GDGT, glycerol dialkyl glycerol tetraethers.

at 90°C (Sprott et al. 1997) and in all halophilic Archaea (Kates 1992) indicates tetraethers are not required for life under extreme conditions. Instead, the taxonomic distribution of archaeal lipids (**Figure 3**) mirrors the evolutionary history of this diverse domain, and it indicates that the tetraether and diether compositions of archaeal membrane lipids are influenced by phylogeny. An early divergence of Thaumarchaeota (Brochier-Armanet et al. 2008, Spang et al. 2010) would suggest that the common ancestor to hyperthermophiles and Thaumarchaeota was a moderate thermophile that was able to synthesize cyclized GDGTs. In the future, the annotation of genes involved in ring formation may shed additional light on the origin of specific GDGTs and illuminate which species among the still-uncharacterized groups of Archaea are also GDGT producers.

The most widely studied physiological correlations for GDGT composition are to growth temperature and pH, with the number of rings increasing as temperature increases and pH decreases (Boyd et al. 2011, Derosa et al. 1986, Macalady et al. 2004, Uda et al. 2001, and references therein; for a review, also see Chong 2010). Hyperthermophiles make GDGTs with up to eight cyclopentane rings. It is not known if mesophiles have the biosynthetic capacity to make GDGTs with more than five rings, but the lack of GDGT-6 to -8 in typical marine sediments suggests that they cannot. Biosynthesis of crenarchaeol is currently thought to be further restricted to the Thaumarchaeota and possibly associated specifically with ammonia oxidation (de la Torre et al. 2008, Pitcher et al. 2010, Schouten et al. 2008a). Interestingly, moderate thermophiles appear to have the highest relative proportion of crenarchaeol (Zhang et al. 2006). For example,

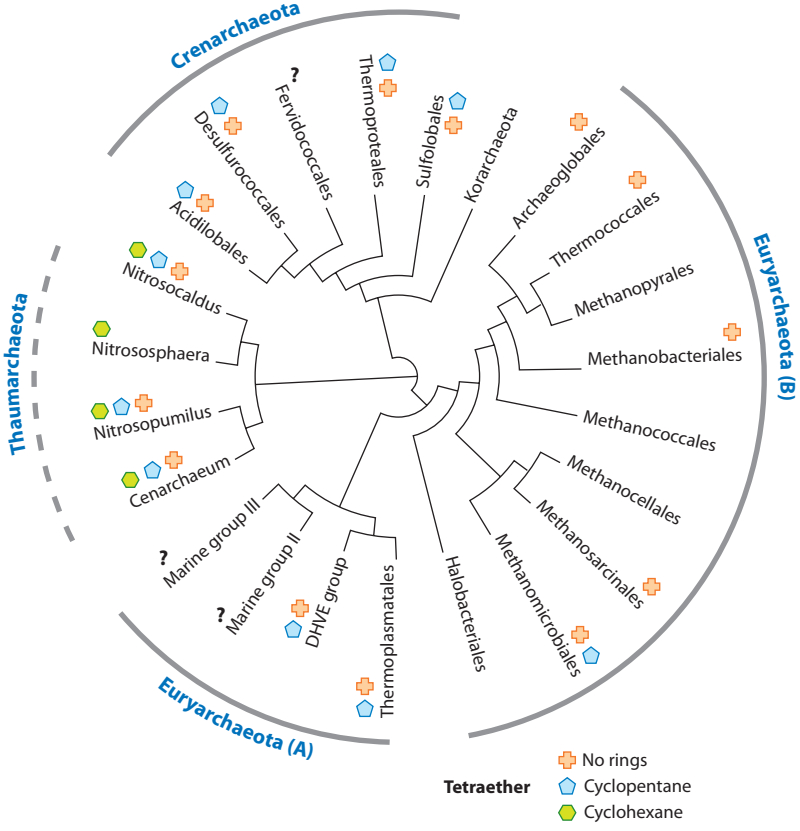

Figure 3

Phylogenetic tree showing the topology of relationships between orders of Archaea based on 16S rRNA sequences. The major kingdoms of Archaea are the Korarchaeota, Crenarchaeota, Thaumarchaeota, and Euryarchaeota. Within the Euryarchaeota, the euryarchaeal group comprising Thermoplasmatales and their relatives (*A*), as well as all Thaumarchaeota and Crenarchaeota studied to date, are known for synthesizing GDGT (glycerol dialkyl glycerol tetraether) lipids containing cyclopentane rings. Only the Thaumarchaeota are known to make crenarchaeol. Abbreviation: DHVE, deep-sea hydrothermal vent Euryarchaeota.

Nitrososphaera gargensis grown at 46°C contains only crenarchaeol and its regioisomer (Pitcher et al. 2010). Environmental surveys and cultured representatives suggest the temperature optimum for the proportion of crenarchaeol relative to GDGT-0 is ~40°C (**Figure 4**), although isothermal variation in this ratio can be induced by varying the growth pH in *Nitrosocaldus yellowstonii* (de la Torre et al. 2008). Understanding how these physiological factors affect crenarchaeol abundance may be significant, as its isomer, cren′, is part of the TEX_{86} paleotemperature index (see below).

Most of the literature on GDGT physiology has focused on how rings promote closer packing of isoprenoid chains in the membrane (Sinninghe Damsté et al. 2002, Gliozzi et al. 1983). However, this closer packing also brings polar head groups closer together (Shimada et al. 2008). Molecular dynamics calculations suggest this increases the strength of hydrogen bonding between neighboring polar head groups, in turn decreasing the permeability of the membrane to solutes, including protons (Chong 2010, Gabriel & Chong 2000). Archaea may place glycosylated GDGTs

TEX_{86}: TetraEther indeX of tetraethers containing 86 carbon atoms

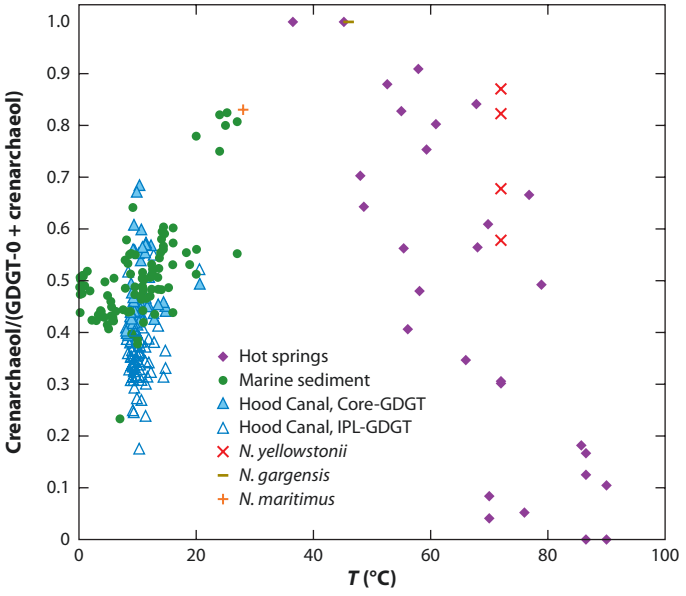

Figure 4

The ratio of crenarchaeol to GDGT-0 reaches a maximum at mid-temperatures in a data set that includes marine and lacustrine systems, hot springs, and cultures of both thermophiles and mesophiles. Data compiled as follows: hot springs (de la Torre et al. 2008, Zhang et al. 2006), marine sediment (Kim et al. 2010), the Hood Canal (Ingalls et al. 2012), *Nitrosopumilus maritimus* (Schouten et al. 2008b), *Nitrosocaldus yellowstonii* (de la Torre et al. 2008), and *Nitrososphaera gargensis* (Pitcher et al. 2010). Abbreviations: GDGT, glycerol dialkyl glycerol tetraether; IPL, intact polar lipid.

on the outer face of their membranes to reduce proton permeability, thereby conserving energy (Shimada et al. 2008, Ulrih et al. 2009).

These studies suggest that synergistic selection of polar head groups and ring-containing side chains could help Archaea acclimate to a variety of environmental stressors including temperature, pH, resource starvation, ionic strength, and pressure. Such adaptations are compatible with hypotheses suggesting that Archaea are optimized to survive in all types of extreme environments, including those with chronically low energy availability such as the open ocean (Valentine 2007). If this is true, however, communities optimized for different nutrient regimes could have different degrees of GDGT cyclization and/or polar head group composition that are related to energy stress rather than strictly to temperature or pH. Such membrane compositional control provides a mechanistic basis for understanding variations in cyclopentyl ring distributions among GDGTs of Archaea in the ocean. It, along with regional variation in archaeal phylogeny, also provides a lens through which we can view the regional and temporal applicability of the TEX_{86} paleothermometer. Future work should focus on understanding the coupling among these many variables.

2.2. The TEX_{86} Index and Initial Calibration to Global Sea Surface Temperature

SST: sea surface temperature

To examine patterns of GDGT cyclization in the marine environment, Schouten et al. (2002) gathered 40 sediment core tops from 15 locations. All were from continental margins, and they encompassed a range of mean annual sea surface temperature (SST) values between 2 and 27°C, salinities between 20 and 35, and oxygen concentrations ranging from anoxic to fully oxygenated.

Such sediments routinely show the following pattern: Cold-temperature samples are dominated by GDGT-0 and crenarchaeol and are relatively depleted in all the minor GDGTs (**Figure 2*a***). By contrast, warm environments have more of all the minor GDGTs (GDGT-1 to -3) and have crenarchaeol:GDGT-0 ratios >1 (**Figure 2*b***). Although qualitatively apparent, how to derive an optimal relationship between GDGT composition and SST remained unclear. Schouten et al. (2002) plotted mean annual SST (as derived from the 1998 World Ocean Atlas) against (*a*) the weighted average number of cyclopentane rings ($r^2 = 0.63$), (*b*) the ratio of GDGT-0/(GDGT-0 + cren) ($r^2 = 0.62$), and (*c*) the ratio of (GDGT-1 + GDGT-2 + GDGT-3 + cren) to total GDGTs ($r^2 = 0.68$). These relationships all demonstrated a positive correlation between the number of rings and SST, but the TEX_{86} (TetraEther indeX of tetraethers containing 86 carbon atoms) ratio resulted in the best statistical correlation:

$$TEX_{86} = \frac{(GDGT\text{-}2 + GDGT\text{-}3 + cren')}{(GDGT\text{-}1 + GDGT\text{-}2 + GDGT\text{-}3 + cren')}. \quad (1)$$

The best relationship to temperature was a linear function:

$$TEX_{86} = 0.015T + 0.28 \quad (r^2 = 0.92). \quad (2)$$

The relationship of TEX_{86} to SST is strictly empirical. A plot of TEX_{86} against the mean annual temperature at 100-m depth also yielded a very similar line with slope = 0.022, intercept = 0.27, and $r^2 = 0.86$. This somewhat poorer correlation was interpreted to suggest that the GDGTs in sediments were more closely tied to biomass produced in surface waters, despite evidence that the greatest production of archaeal biomass may be in subsurface waters (Karner et al. 2001, Massana et al. 1997).

Using only the minor GDGTs in the TEX_{86} equation was critical to optimize the correlation between cyclopentane rings and temperature. Because GDGT-0 is common in gut- and sediment-dwelling methanogens as well as other Euryarchaeota, eliminating GDGT-0 was thought to remove their influence. However, given the probability that Group II planktonic Euryarchaeota also produce GDGT-0 to -3 (see Section 3), this may be a moot argument. The primary benefit of removing GDGT-0 and crenarchaeol from the TEX_{86} equation is mathematical: The variance of small relative changes in high-abundance compounds swamps the signal of small relative changes in the low-abundance compounds. (Note, however, an alternative approach to this problem may be to divide the abundances of GDGT-0 and crenarchaeol by an empirically derived constant.)

Although most core top-derived TEX_{86} values fall on or close to the global calibration, some locations show large deviations ($>1\sigma$) from the original line. More generally, there has been debate about which form the TEX_{86} equation and calibration(s) should take. At least four reasons for these uncertainties arise: (*a*) questions about the mathematical form of the TEX_{86} relationship, especially at high and low temperatures; (*b*) the potential influence of sediment transport or degradation processes, including terrestrial GDGT inputs in some locations; (*c*) the role of different ecological communities of Archaea in producing exported GDGTs; and (*d*) spatial and temporal heterogeneity in GDGT sources in the water column (e.g., depth of GDGT production and export, changes in mixed layer or other water column temperature gradients, and seasonality of archaeal growth). We address these points in the following four sections, with greater emphasis on the vast complexities associated with evaluating the latter two.

3. THE EVOLVING CALIBRATION: LINEAR VERSUS NONLINEAR TEX_{86}

In an early attempt to reconstruct SSTs from the Cretaceous, sediments returned TEX_{86} values as high as 0.97 (Schouten et al. 2003a). Given Equation (2), the resulting SST would be 47°C,

much higher than is physically plausible for the ocean. Although degradation of the original lipids is a possible explanation, core GDGTs generally degrade proportionally, with little alteration of TEX_{86} values (Huguet et al. 2009, Schouten et al. 2004). Notably, the original calibration (Schouten et al. 2002) contained no TEX_{86} values above 0.8. To remedy this inconsistency, researchers calculated a new calibration line based on the observation that samples from warm environments appeared to have a lower slope than those from colder environments. Including only the data from locations with temperatures >20°C, a recalculated high-temperature calibration line with lower slope resulted in Cretaceous SST estimates of 32–36°C (Schouten et al. 2003a).

Although this approach yielded reasonable temperatures, some recent work based on expanded data sets does not support a different slope for temperatures >20°C (Kim et al. 2008a, 2010). Kim et al. (2008a) reported a new global calibration for 287 core top samples, and the TEX_{86} values were compared against temperatures from *World Ocean Database 2001* (Conkright et al. 2002). The data suggested that samples from the Red Sea and those from locations with SSTs <5°C do not have the same linear relationship as the remainder of the ocean. After removing the Red Sea data, along with any additional outliers (n = 24) as defined by samples that were not within ±1σ of the initial correlation line, the resulting 223 filtered samples (a loss of 22% of the available data) resulted in the following relationship:

$$\mathrm{SST} = -10.78 + 56.2 \times \mathrm{TEX}_{86} \quad (r^2 = 0.94). \tag{3}$$

In contrast with this argument for a linear relationship, Liu et al. (2009) used all 287 samples (no filtering for outliers) to plot the reciprocal of TEX_{86}, emphasizing that the TEX_{86} formula describes the change in fractional abundance of GDGT-1 relative to the sum of the other minor GDGTs:

$$\frac{1}{\mathrm{TEX}_{86}} = \frac{\mathrm{GDGT\text{-}1}}{(\mathrm{GDGT\text{-}2} + \mathrm{GDGT\text{-}3} + \mathrm{cren}')} + 1. \tag{4}$$

When this reciprocal function was fitted with a straight line, the statistical significance was as good as that found for the unfiltered data of Kim et al. (2008a), and the resulting equation—when plotted as SST versus TEX_{86}—describes a curve:

$$\mathrm{SST} = 50.48 - 16.33\frac{1}{\mathrm{TEX}_{86}} \quad (r^2 = 0.82). \tag{5}$$

When data from all locations are considered, curved and linear functions are equally able to fit the data. Thus, the absolute uncertainty in the global calibration is likely greatest at the high- and low-temperature extremes (**Figure 5*a***).

4. HETEROGENEOUS SOURCES AND PROCESSES: NONPLANKTONIC INPUTS

Other lessons about the calibrations were learned from regions exhibiting mixed sources of GDGTs. For Arctic sediments from the Paleocene/Eocene thermal maximum, Sluijs et al. (2006) concluded that unusually high concentrations of GDGT-3 were attributed to terrestrial inputs. Because excess GDGT-3 caused elevated estimates of SST (21–25°C), GDGT-3 was removed from the original TEX_{86} equation and the new formulation was called TEX_{86}'. The recalibrated relationship yielded SST estimates of 17–21°C. Although terrestrial material did likely alter the GDGT profiles in that location at that time, other environmental changes included increases in stratification and the extent of anoxia. The concomitant effects of such changes on TEX_{86} values remain unknown.

More generally, this example points to the challenge of how to identify sediments that contain archaeal GDGTs only of marine planktonic origin. There are many potential taxonomic and

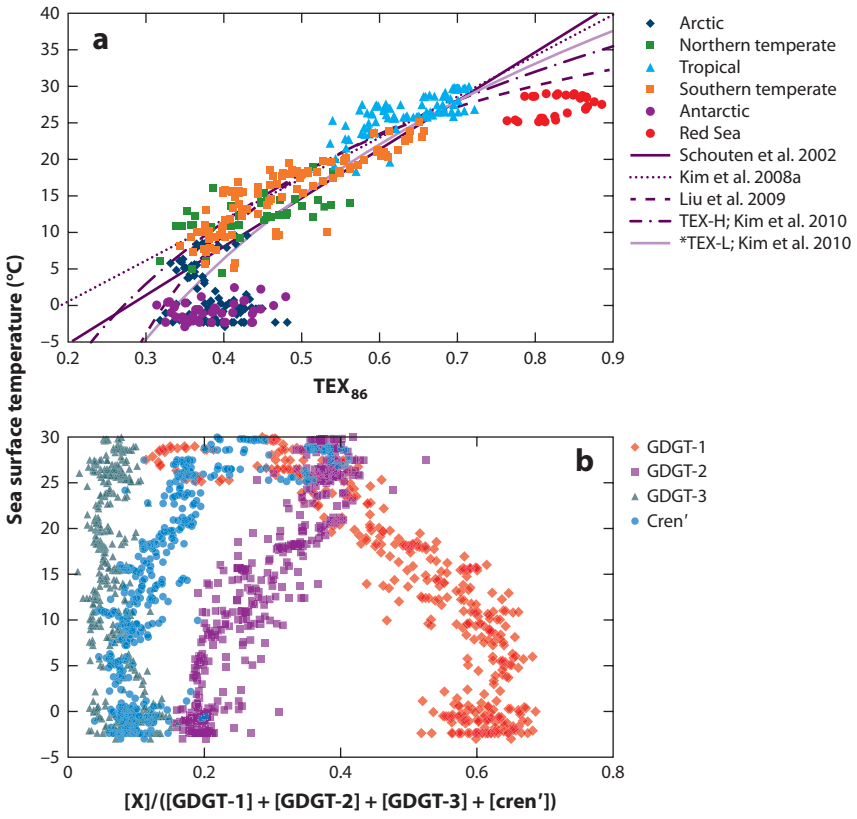

Figure 5

(*a*) TEX_{86} and SST (satellite) data from Kim et al. (2010). Locations have been separated by latitude. Tropical samples are defined as all those between the Tropic of Cancer and the Tropic of Capricorn (23.5°N/S latitude), Arctic data as all those above 55°N, and Antarctic data as all those below 50°S. Northern and Southern Hemisphere data in temperate latitudes also have been distinguished, and the Red Sea is considered separately. All the major TEX_{86}-SST calibration lines from the literature also are shown. [*TEX-L (GDGT index-1) and TEX_{86} do not use the same suite of GDGT compounds. To convert between them, we plotted all data as GDGT index-1 versus TEX_{86} and fit them with the equation GDGT-1 $= 0.571ln(TEX_{86}) - 0.0776$. This equation was then substituted into Equation (7) to plot SST versus TEX_{86} for the TEX-L line.] (*b*) Patterns of individual GDGTs in the underlying data are shown as the proportion of a given GDGT relative to the total of the compounds used in the TEX_{86} index. All data are shown and are not indexed by latitude. Abbreviations: GDGT, glycerol dialkyl glycerol tetraether; SST, sea surface temperature; TEX_{86}, TetraEther indeX of tetraethers containing 86 carbon atoms.

ecological sources of GDGTs (**Figure 3**) (Section 6). The abundance of Thaumarchaeota in soils (Leininger et al. 2006), hot springs (de la Torre et al. 2008, Hatzenpichler et al. 2008), and lacustrine environments (Herrmann et al. 2009, Kim et al. 2008b) implies that GDGTs may be produced universally in nearly all environments. Evidence shows these terrestrial Thaumarchaeota produce the same suite of lipids as marine planktonic species, including crenarchaeol.

In addition to terrestrial influences, at least two other cases require additional caution. The first is in situ production in sediments by organisms involved in methane cycling (Zhang et al. 2011). Anaerobic methane-oxidizing division ANME-1 appears to contain GDGT-0 to -4 (**Figure 3**) (Blumenberg et al. 2004, Thiel et al. 2007), and methane-rich sediments have unusual

GDGT distributions with anomalously negative $\delta^{13}C$ values due to these sources (Pancost et al. 2001, Schouten et al. 2003b). To date, it remains controversial whether additional GDGTs are contributed by other benthic Archaea in addition to methanogens and methanotrophs. The IPL forms of the GDGTs (IPL-GDGTs) are abundant in marine sediments (Lipp et al. 2008), but this may reflect a relatively slow degradation rate rather than the presence of actively growing cells (Schouten et al. 2010). Recent experiments have suggested that, even among the living fraction of sedimentary Archaea, some of their isoprenoids, core lipids, or partially degraded core lipids (glycerol dialkyl diols) may be recycled from the relict planktonic input (Liu et al. 2012, Takano et al. 2010). If so, the net TEX_{86} may be minimally affected.

The second cautionary tale concerns cren′, an enigmatic compound that can be nearly undetectable in cultures and enrichment mesocosms of Thaumarchaeota as well as in suspended particulate matter collected from marine surface waters (Ingalls et al. 2006, Wuchter et al. 2004). [However, a major exception is its high abundance in the thermophile *N. gargensis* (Pitcher et al. 2010).] In contrast, cren′ is commonly present at up to 15% of the crenarchaeol concentration in sediments. Shah et al. (2008) reported a significantly "older" ^{14}C content of cren′ relative to crenarchaeol in Santa Monica Basin sediments, suggesting a possible benthic contribution to the cren′ pool and/or different (but nonplanktonic) processes contributing to its production and sedimentation. More evidence is needed to determine whether these alternate sources are widespread.

5. REGIONAL HETEROGENEITY: EFFECTS OF COMMUNITY AND GEOGRAPHY?

The power of global data sets of GDGT distributions may be best harnessed to examine individual environments within the global context. Such analyses may indicate the circumstances for which local calibrations would be preferable. Several such cases are currently under study.

5.1. High- and Low-Temperature Extremes

Values of TEX_{86} from the Red Sea predict temperatures that are much higher than local observations of SST (Trommer et al. 2009). Ionescu et al. (2009) definitively demonstrated in this case that there are taxonomic groups of Archaea in the Red Sea that are phylogenetically distinct from open-ocean species. Trommer et al. (2009) speculated that the high salinity of the Red Sea could play a role in altering the GDGT composition of the local community. Their study demonstrates that distinct marine populations can have divergent TEX_{86} signatures, and consistent with results from hot springs and cultures of thermophiles, it is another example demonstrating that GDGT lipids from very-warm-temperature systems do not fall on global marine calibration lines for TEX_{86} (Pearson et al. 2004, Pitcher et al. 2009, Uda et al. 2001).

Examining the other temperature extreme, Shevenell et al. (2011) applied TEX_{86}-based temperature reconstructions to a polar region, focusing on Holocene records from the Palmer Deep near Antarctica. When the authors used all of the existing calibration curves (Kim et al. 2008a, 2010; Liu et al. 2009), they found that reconstructed SST values showed considerable scatter and included predicted SST values as low as −20°C, which is well below the freezing point of seawater. To compensate, they used local core top data to generate a modern, regional calibration for SST. When these data were applied to the down-core record, Holocene temperatures were predicted to be in the reasonable range of −2 to 6°C. Accordingly, employing local calibrations taken from geographically constrained systems may be more fruitful than attempting to apply a single global calibration to all locations.

5.2. Global Low-Temperature Patterns

Even beyond the extremes of very hot or very cold marine regions, evidence indicates that the global calibration obscures other regional trends. In some cases, deviations from the global calibration are of a relatively small magnitude such that they could go unnoticed within the global data unless specifically highlighted. Recognizing the strong interest in polar regions, Kim et al. (2010) developed a TEX_{86} equation optimized for lower-temperature environments. The global data set (excluding the Red Sea) was examined in an attempt to overcome the poor correlation observed at low temperatures (**Figure 5*a***). The result was GDGT index-1, which is log(TEX_{86}) after removing GDGT-3 from the numerator and eliminating cren′ entirely:

$$\text{GDGT index-1} = \log\left[\frac{\text{GDGT-2}}{(\text{GDGT-1} + \text{GDGT-2} + \text{GDGT-3})}\right]; \quad (6)$$

$$\text{SST} = 67.5\,(\text{GDGT index-1}) + 46.9 \quad (r^2 = 0.86, n = 396). \quad (7)$$

GDGT index-1, also called TEX_{86}^{L}, includes all core top data available (now 396 core tops). The authors specify the correlation is useful in the temperature range −3 to 30°C (note that −3°C is below the freezing point of surface seawater).

The proposed explanation for the improved correlation between GDGT index-1 and low SSTs is that cren′ plays a smaller role in temperature adaptation in cold environments as evidenced by the lack of correlation between the relative abundance of cren′ with SST below ∼10°C (**Figure 5*b***). Both in mesocosm experiments (Wuchter et al. 2004) and in surface waters (Ingalls et al. 2006), cren′ is present in very low abundance—but this observation applies at all temperatures, including at >10°C. In addition, there is similarly poor correlation of GDGT-1 and GDGT-2 with SST in the low-temperature range, whereas GDGT-3 shows minimal trend at any temperature (**Figure 5*b***).

Such patterns are not random: Close examination of the data reveals regional trends in the overall TEX_{86} values (**Figure 5*a***) as well as in the relative contribution from specific GDGTs. Using cren′ abundance (relative to crenarchaeol) as an example, we find two clusters of points in the data below 10°C, one significantly above the mean of the data set and one below (**Figure 6**). All the Antarctic data are in the lower cluster and form a line of low slope but good correlation ($r^2 = 0.59$) (**Figure 6**). For the Arctic, some data are in the upper group, whereas others are interspersed with the Antarctic data. However, removing all the Arctic data with SSTs below 1°C (many of which are below the freezing point of surface sea water) would eliminate all these low values, leaving the remaining Arctic data to define a line of steeper slope. Polar locations may support communities of Archaea with fundamentally different GDGT compositions for a given temperature relative to other locations in the global calibration, much as observed for the Red Sea. Local communities could include sea ice Archaea with different GDGT signatures, lateral transport of particle-associated biomass, or large contributions of terrestrial organic matter. The latter point could be assessed from contributions of terrestrial GDGTs (Hopmans et al. 2004, Sluijs et al. 2006).

It may also be relevant that much of the data falling below the line in the global calibration (**Figures 5*a*** and **6**) has satellite-assigned SSTs of −2.4°C, which is below the freezing point of seawater. Such temperatures cannot be the actual in situ temperatures for growth of Archaea in the sea surface. Substituting a higher temperature would move these points closer to the other data but would not change the overall pattern of difference between the Arctic and Antarctic samples.

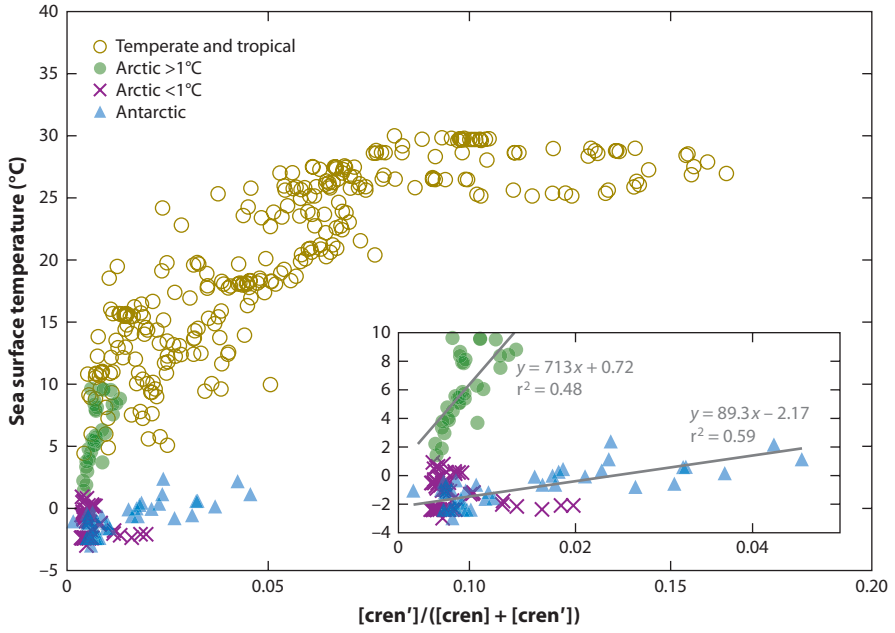

Figure 6

An example of regional patterns in GDGT ratios. The proportion of crenarchaeol regioisomer (cren′) relative to crenarchaeol drops off precipitously at cold temperatures. However, within the data from locations with SST <10°C, the Arctic and Antarctic regions are different. These differences may be related to temperature (all data <1°C follow a similar trend), or they may be related to geography (Arctic data >1°C show a different trend from Arctic data <1°C). Data are compiled from Kim et al. (2010). Abbreviations: GDGT, glycerol dialkyl glycerol tetraether; SST, sea surface temperature.

5.3. Global High-Temperature Patterns

In a similar effort to examine regional effects and to improve reconstructions at the high end of the temperature spectrum, Kim et al. (2010) proposed a second GDGT index specific for high temperatures. Termed the GDGT index-2, this is the logarithm of the traditional TEX_{86} equation:

$$\text{GDGT index-2} = \log(TEX_{86}). \quad (8)$$

GDGT index-2 yields the following calibration equation:

$$SST = 68.4(\text{GDGT index-2}) + 38.6\,(r^2 = 0.87,\ n = 255). \quad (9)$$

Additionally, its use is recommended only for temperatures above 15°C. This is known as the TEX_{86}^{H} (TEX_{86}-high) calibration. The authors recommend TEX_{86}^{L} (TEX_{86}-low), because it must be used for records with temperatures below 15°C and for any record that encompasses both temperature ranges (Kim et al. 2010). Note, however, that the 15°C cutoff is arbitrary. If the cutoff were imposed as in **Figure 5*a***, where tropical samples are separated from temperate samples at 23.5°N/S latitudes, the temperature at this boundary would be closer to 20°C and the apparent slope of the tropical calibration would be slightly shallower than that defined by Equation (9).

5.4. Experimental Approaches to Understanding Nonconforming Locations

To understand how the GDGT composition of an archaeal community adjusts in response to environmental conditions, it is necessary to understand the relationship to physical variables, including—but not limited to—temperature. There are three main approaches: mesocosm incubations, culture studies, and analysis of GDGTs in water column filtrates or sediment trap samples. Experiments on pure cultures are ideal, but only one GDGT-producing mesophile that grows readily in pure culture, *Nitrosopumilus maritimus* (Könneke et al. 2005), has been isolated to date. Attempts to use *N. maritimus* to test the relationship between TEX_{86} and growth temperature have met with challenges because the organism grows optimally only in a narrow temperature range (25–30°C) in the laboratory (D. Stahl, personal communication).

An alternative to pure-culture techniques is the use of enrichment mesocosms. The first attempt to study large mesocosms yielded some surprises (Wuchter et al. 2004). Even though the basics of the temperature response—more cyclopentane rings in samples harvested at higher temperatures—were observed, the community of Archaea produced far less of the crenarchaeol regioisomer than normally detected in sediment core tops. This dearth of cren′ resulted in a TEX_{86} versus temperature line that had the same slope as the original calibration (Equation 2), but with a lower intercept. The reason for the low abundance of cren′ remains a mystery. Clues may be found in the disparate GDGT distributions of isolates and enrichment cultures of Thaumarchaeota. *N. maritimus*, like the mesocosm, contains only very small proportions of cren′ (Schouten et al. 2008b), as does the symbiont *Candidatus* Cenarchaeum symbiosum (Sinninghe Damsté et al. 2002). In contrast, the thermophiles *N. yellowstonii*, especially *N. gargensis*, appear to contain abundant cren′ (de la Torre et al. 2008, Pitcher et al. 2010).

In the mesocosm studies, core GDGTs were analyzed without prior hydrolysis of the polar head groups of their IPL-GDGTs. Measurements that are based on core GDGTs can be challenging to interpret, because IPL-GDGTs are the form present in living cells, whereas core lipids are formed after cell death (Harvey et al. 1986). The low concentrations of core GDGTs at the initial time point likely existed alongside a large—but not analyzed—pool of intact GDGTs. Because of the lag in degradation of IPL-GDGTs after cell death, the core GDGTs detected at later time points of the mesocosms may have represented GDGTs produced weeks prior to the time points analyzed. Thus, cren′ might have been produced in the incubations, but its polar head groups were more robust. However, this seems implausible given the likelihood that two isomers of IPL-GDGTs would have similar stability. To address these issues, future mesocosm or culture experiments should analyze the GDGTs associated with the living fraction of archaeal biomass.

Another possible explanation for the lack of regioisomer is that the cells that produce this compound were not collected on the filters used for sampling. Additionally, the abundance of this GDGT may have been low enough that it was not within the linear range of the mass-spectrometric detection method. The same challenges apply to studies of water column particulate matter. In the mesocosms, 100 ml of water was filtered through a 0.7-μm glass fiber filter (Wuchter et al. 2004). Nearly all water column environmental samples also are collected on 0.7-μm glass fiber filters and generally have reported core GDGTs, rather than IPL-GDGTs. This particle-size cutoff excludes many of the Bacteria existing in the 0.2–0.7-μm size range (approximately 40–70% of bacterial cells) (Altabet 1990, Koike et al. 1990) and has been demonstrated to miss a significant fraction of the Archaea as well (Ingalls et al. 2006, 2012). Although core lipid abundance profiles in the water column can sometimes mirror those of IPL-GDGTs, there is not an overall good correlation between the sizes of the two pools (Huguet et al. 2010), nor is there agreement between the relative abundances of compounds in the two pools (A.E. Ingalls, unpublished data). We recommend that more studies should employ comprehensive hydrolysis (e.g., Ingalls et al. 2006) and/or characterize

both the IPL and core GDGT fractions (Ingalls et al. 2012, Lipp & Hinrichs 2009, Liu et al. 2011, Pitcher et al. 2011a).

Even though enrichment or culture-based calibration studies may not be directly applicable for paleoreconstruction, having numerous experiments at a variety of temperatures and environmental conditions may help us to understand how single species regulate their membranes. A better understanding of regional and community variations in TEX_{86} requires both more knowledge of the physiological basis of GDGT distributions and a better ecological understanding of the niche space of marine Archaea.

6. FACTORS AFFECTING GDGT PRODUCTION AND EXPORT

Group I cells typically are the dominant Archaea in the water column. Finer taxonomic distinction now parses this division into Groups I.1a, I.1b, and I.1c: Group I.1a is predominant in aquatic systems, whereas Groups I.1b and I.1c are dominant in soils and other terrestrial systems (DeLong 1998, Jurgens et al. 2000, Ochsenreiter et al. 2003). The physiological distinctions between these subgroups remain largely unknown. The majority of all Group I Archaea are believed to be free living rather than attached to particles (DeLong et al. 1993, Ingalls et al. 2012); little is known about Group II or III Euryarchaeota, although a genome was recently reported for the Group II division (Iverson et al. 2012).

All Thaumarchaeota are presumed to be potential sources of GDGTs to the environment (**Figure 3**). Great unknowns remain regarding how to forecast differences in GDGT lipid profiles between taxonomic subgroups and how to factor in the potential contribution of GDGTs from Euryarchaeota. A better understanding of the physiology of marine Archaea as well as their diversity and ecology promises to help researchers interpret TEX_{86} lipid ratios.

6.1. Thaumarchaeota: Diversity and Metabolism

The planktonic, lacustrine, and soil-dwelling Thaumarchaeota known to date are primarily aerobic, ammonia-oxidizing chemoautotrophs. The first hint at widespread autotrophy came from $\delta^{13}C$ values of the C_{40} isoprenoid side chains of GDGTs. Values ranging from -21 to $-23‰$ indicated a heterotrophic diet specific for ^{13}C-enriched substrates or a non-RuBisCO-dependent, chemoautotrophic lifestyle (Hoefs et al. 1997). Potentially controverting evidence soon followed: Planktonic Archaea were found capable of taking up amino acids, leading to the suggestion that they are partially heterotrophic or mixotrophic (Ouverney & Fuhrman 2000). The overall metabolic balance of marine communities, however, appears to lie on the side of autotrophy. The natural radiocarbon (^{14}C) content of C_{40} isoprenoids and GDGTs recovered from marine sediments (Pearson et al. 2001, Shah et al. 2008) and the water column (Ingalls et al. 2006) shows values consistent with incorporation of dissolved inorganic carbon (DIC) into biomass, although the latter study also predicted approximately 20% incorporation of organic carbon via heterotrophy or mixotrophy. Similarly, a ^{13}C-labeling study showed incorporation of ^{13}C-DIC into GDGTs at a rate far exceeding ^{13}C enrichment of bacteria during the same incubation, again implying a dominantly autotrophic source for biomass carbon of planktonic Archaea (Wuchter et al. 2003).

Thus far, pure and enrichment cultures of Thaumarchaeota have been consistent with these interpretations. Although not amenable to active culturing, the first named species (*Candidatus* Cenarchaeum symbiosum) was identified as a symbiont of a marine sponge (Preston et al. 1996). Its GDGT lipid profile (Sinninghe Damsté et al. 2002, DeLong et al. 1998) and genome (Hallam et al. 2006) subsequently verified both the presence of crenarchaeol and the presence of

genes for autotrophic oxidation of ammonia. The eventual "smoking gun" was the experimental characterization of a pure culture, *N. maritimus*, an obligate ammonia-oxidizing chemoautotroph (Könneke et al. 2005, Martens-Habbena et al. 2009, Walker et al. 2010). To date, it remains the only species readily grown in axenic culture. Other pure strains have been obtained in coculture with Bacteria: *N. yellowstonii* (de la Torre et al. 2008); *N. gargensis* and *Nitrososphaera viennensis* (Hatzenpichler et al. 2008, Tourna et al. 2011); *Nitrosotalea devanaterra* (Lehtovirta-Morley et al. 2011); *Nitrosoarchaeum koreensis* and *Nitrosoarchaeum limnia* (Blainey et al. 2011, Kim et al. 2011); Group I.1a strains CN25, CN75, and CN150 (Santoro & Casciotti 2011); and *Nitrosopumilus salaria* (Mosier et al. 2012).

***amoA*:** ammonia monooxygenase gene, subunit A

Metabolic characterization shows that all these cultures oxidize ammonia and make the characteristic GDGT crenarchaeol (**Figures 1** and **3**), despite origins from a wide diversity of physical environments (soils, marine systems, hot springs) and growth conditions (<10 to 74°C, pH 4.5 to >8.0, variable salinity). Induction and maintenance of an axenic culture of *N. viennensis* were achieved—albeit with great difficulty—by adding excess pyruvate and/or sterile supernatant from the original enrichment, suggesting that, unlike the unique case of *N. maritimus*, most Thaumarchaeota at least partly depend on bacterial cometabolites (Tourna et al. 2011). In addition, it is likely that not all Thaumarchaeota are ammonia oxidizers, as an anoxic habitat devoid of ammonia [and a genome lacking *amoA* (archaeal ammonia monooxygenase)] (Nunoura et al. 2011) would appear to preclude this metabolism; it is unknown whether non-ammonia-oxidizing species also lack crenarchaeol.

6.2. Thaumarchaeota: Distribution in the Water Column

Because oxidation of ammonia may be a unifying metabolism for aerobic Thaumarchaeota (Pester et al. 2011), the abundance and activity of this group are commonly enumerated by counting *amoA* genes and mRNA transcripts as well as 16S rRNA genes. Such approaches lead to estimates of cell numbers and, importantly, also identify the environmental zones of maximum metabolic activity. Group I Thaumarchaeota are ubiquitous in marine waters, including polar regions, coastal and estuarine environments, oligotrophic oceans, eutrophic environments, and suboxic zones (Francis et al. 2005, Karner et al. 2001, Lam et al. 2007, Massana et al. 1997, Mincer et al. 2007, Murray et al. 1998).

Such ubiquity suggests that Archaea play a significant role in the marine nitrogen cycle (Francis et al. 2005, Wuchter et al. 2006a). Genes and/or transcripts for *amoA* are found in both oxic and suboxic water columns, and quantitative enumeration shows Archaea outnumber bacterial ammonia oxidizers by at least an order of magnitude (Agogué et al. 2008, Church et al. 2010, Lam et al. 2007, Mincer et al. 2007, Urakawa et al. 2010). The explanation for the success of Thaumarchaeota is likely kinetic: *N. maritimus* is optimized for nutrient-limited conditions. It has a half-saturation constant (K_m) of only 100 nM ($NH_3 + NH_4^+$), which is 1,000-fold lower than the average K_m for ammonia-oxidizing bacteria (Martens-Habbena et al. 2009). This value is suitable for the low ammonia concentrations of the open ocean and suggests that Archaea can keep the concentration of ammonia low enough to outcompete Bacteria. They may also be able to compete with some phytoplankton for ammonia in the photic zone, as is suggested by measureable nitrification rates in shallow waters (Yool et al. 2007).

Environmental factors dictate the vertical distribution of these Archaea in the water column. Very low numbers are common in the upper photic zone, which may be due primarily to photoinhibition and competition for regenerated ammonia (Ward 1985). However, other unexamined mechanisms, including competition for other scarce resources (e.g., trace metals), the presence

of other inhibitors, or high rates of biomass loss to grazing or sinking, could be at play. In most environments, gene abundances increase rapidly with depth, with maximum copies of *amoA* and 16S rRNA near the base of the photic zone (Church et al. 2010, Francis et al. 2005, Karner et al. 2001, Massana et al. 1997, Santoro & Casciotti 2011). The number of mRNA transcripts per cell appears to be highest in the photic zone, but the total number of mRNA transcripts is highest at its base, in accordance with the zone of maximum archaeal cells (Church et al. 2010). Below the photic zone, cell abundances remain high, but transcript abundance slowly tapers off with depth, suggesting a less active population. The region between the base of the photic zone and the oxygen minimum zone is a long-recognized zone of nitrification and nitrite accumulation (Lipschultz et al. 1990, Ward 1987). Accordingly, maximum abundances of Group I Thaumarchaeota also are coincident with nitrite maxima. The constant ~1:1 ratio of *amoA*:16S rRNA genes also shows that the majority of Thaumarchaeota in the upper 1,000 m of the water column possess ammonia-oxidizing cellular machinery. Previous reports that this ratio decreases with depth (Agogué et al. 2008) are proposed to be an artifact of PCR primer mismatch (Church et al. 2010).

6.3. Thaumarchaeota: Physiological and Seasonal Implications for TEX_{86}

Determining the depth in the water column in which the majority of archaeal GDGTs are produced is of paramount importance for researchers to understand the sources of these compounds to marine sediments. If we assume *amoA* transcripts are proportional to activity, new autotrophic biomass will primarily be formed at or near the base of the photic zone (Church et al. 2010). Such activity estimates are consistent with older data that show the majority of ammonia oxidation associated with inorganic carbon fixation in the open water column occurs between 150 and 550 m (Karl et al. 1984). In turn, the quantitative majority of GDGT production by Thaumarchaeota also occurs at these depths. Importantly, this region is home to the largest environmental gradients in the ocean, namely the thermocline and chemocline. Factors that influence how and where Archaea grow in relationship to these physical gradients may affect interpretations of paleoproxies that are based on GDGTs.

Given that the deep-photic to mesopelagic environment is the likely source of most archaeal biomass, a direct relationship between thermocline temperature and other physiological variables may exert much of the control over GDGT composition. If the thermocline temperature is offset from SST in a predictable way, such that the temperature at depth of maximum archaeal growth always remains well correlated with SST, then this autocorrelation relationship would explain why the calibration of TEX_{86} to SST works. The TEX_{86} value will correlate with both the in situ growth temperature and SST. Slightly poorer correlation coefficients of TEX_{86} ratios to, e.g., 100-m or 200-m temperatures may be due to the local steepness of temperature gradients and variability in the depth of the thermocline. Data points in the global TEX_{86} calibration that do not fall on the global average SST correlation line could be explained as locations in which the relationship between SST and thermocline temperature is unstable or disrupted by unusual circulation patterns. Water column structure in the Antarctic may be especially vulnerable to this effect (Section 5.2).

To develop GDGT-based paleoproxies, investigators must know the depth of GDGT production and be able to constrain the timing of production and processes of export of GDGTs. Both the spatial and temporal distributions of phylogenetic groups, and the subsequent processing of their GDGT signals, are factors in determining final TEX_{86} ratios in sediments. The seasonality of archaeal production remains relatively unknown because it is usually judged by 16S gene or GDGT core lipid abundances rather than rates of archaeal biomass production. However, assuming that

cell abundances correlate with production rates, then winter production appears to be the favored season. This is supported by data from the Southern Ocean, Arctic Ocean, Mediterranean Sea, and North Sea (Alonso-Sáez et al. 2008, Galand et al. 2010, Manganelli et al. 2009, Murray et al. 1998, Pitcher et al. 2011b). The physiological argument for this pattern centers on competition for ammonia. Archaea may use ammonia generated by the decay of phytoplankton biomass, favoring out-of-phase seasonality, with Archaea lagging phytoplankton (Herfort et al. 2007, Murray et al. 1998). Additional evidence suggests that over geological timescales, GDGT concentrations scale with indicators of productivity; thus, the flux is maximized when the net system productivity is highest (Fietz et al. 2011, Shevenell et al. 2011). However, if most archaeal biomass is produced near or in the thermocline, any seasonal effects on temperature may be severely diminished by lack of variability in these deeper waters.

A final caveat to these discussions arises from evidence suggesting that not all planktonic Thaumarchaeota are strict autotrophs. Such functional diversity may be important, especially if it is linked to GDGT profiles via the hypothesized mechanism of controlling the number of rings to maintain cellular energy balance (Section 2.1). Just as cultures of hyperthermophilic Crenarchaeota have different rings versus temperature relationships (Uda et al. 2001) compared with those of enrichment cultures of ammonia-oxidizing Thaumarchaeota (Schouten et al. 2007, Wuchter et al. 2004), functionally different groups within the Thaumarchaeota may have different ring versus temperature relationships relative to each other. Mixotrophic and/or heterotrophic metabolisms in natural populations are indicated by the ability to take up amino acids (Ouverney & Fuhrman 2000) and by a natural ^{14}C content that does not strictly correspond to ^{14}C-DIC (Ingalls et al. 2006). The *N. maritimus* genome contains a partial tricarboxylic acid cycle and numerous transporters for small organic substrates, potentially enabling mixotrophy in this species and its relatives (Walker et al. 2010). Recently, researchers suggested that some Group I.1b (soil-type) Thaumarchaeota enriched in a wastewater facility lacked sufficient rates of ammonia oxidation and carbon fixation to account for their cell numbers, leading to speculation that they can grow as facultative heterotrophs, although heterotrophic activity was not directly demonstrated in this system (Mussmann et al. 2011).

6.4. Thaumarchaeota in Sediments

Other novel groups also have been reported from marine sediments and hot springs, the most common of which appear to be the Marine Crenarchaeota Group, Marine Benthic Group D, and Hot-Water Crenarchaeota Group. The sedimentary groups have unknown physiological affinity (Biddle et al. 2006, Nunoura et al. 2011, Takano et al. 2010), and only the hot-water group has any representatives in culture (*Nitrosocaldus* sp.) (de la Torre et al. 2008). However, some classification methods suggest that all these groups may be members of the Thaumarchaeota (Pester et al. 2011; K. Lloyd, personal communication).

Among the sedimentary species, some may be neither ammonia oxidizers nor primarily autotrophs. Lack of O_2 or *amoA* genes (Nunoura et al. 2011) would appear to preclude a lifestyle of ammonia oxidation, although, in the former circumstance, the needed O_2 equivalents potentially could be generated from nitrite (Ettwig et al. 2010); the resulting metabolism would be thermodynamically similar to anammox. However, GDGTs recovered from deep, anoxic sediments suggest that the dominant metabolism for Archaea living without access to O_2 is anaerobic heterotrophy (Biddle et al. 2006, Lin et al. 2010). One possible source of new GDGTs to sediments would be from these in situ heterotrophs. Recent evidence suggests that members of the Marine Crenarchaeota Group (now Thaumarchaeota) are metabolically distinct from Group I Thaumarchaeota (Li et al. 2012).

There also appear to be links between the Thaumarchaeota and the sulfur cycle. A sediment-dwelling relative of *N. maritimus* grew better in a low-O_2 environment created by coculturing with thiosulfate-oxidizing bacteria (Park et al. 2010). Similarly, very large cells of Thaumarchaeota (genus *Giganthauma*) from a mangrove swamp were found covered with sulfur-oxidizing Gammaproteobacteria (Muller et al. 2010). Although the suggested symbiosis in this case was sulfide detoxification, an additional role may be O_2 depletion and/or stimulation of ammonia oxidation. These findings suggest that certain strains of Thaumarchaeota may be favored at redox boundaries. For further review, see Pester et al. (2011).

6.5. Euryarchaeota

Little is known about the ubiquitous Group II and III Euryarchaeota other than that they are present in low abundance in marine pelagic environments, where they are usually outnumbered by Group I Thaumarchaeota. However, Group II Euryarchaeota are usually the dominant phylotype in surface waters (Delong 1992, Massana et al. 2000) and can be especially important in certain niches (Martin-Cuadrado et al. 2008). In the North Sea, Group II cells outnumber Group I cells by two orders of magnitude, with a preference for summer seasonality (Herfort et al. 2007, Pernthaler et al. 2002). By contrast, off the shore of Hawai'i, Group II and Group I cells are in approximately equal abundance at the surface (Karner et al. 2001). Time-series analysis of the Mediterranean further shows that although Group II.a dominates in summer, Group II.b prefers winter (Galand et al. 2010).

There are currently no Group II or III representatives in culture, but a recently completed genome of Group II—assembled from an environmental metagenome—has revealed some clues about physiology and lipid biosynthesis (Iverson et al. 2012). The lack of gene annotations for ring formation in GDGTs leaves us with no knowledge of the predicted lipid profiles of these organisms, but it is generally assumed that this group may contribute GDGT-0 (which is common in Euryarchaeota) (**Figure 3**) but does not contribute GDGT-1 to -3, crenarchaeol, or its isomer.

We suggest that the latter interpretation is most likely false: Multiple lines of evidence suggest these Euryarchaeota are potential producers of GDGT-1 to -3. Despite lack of cultivation, the taxonomic affiliation of Group II has been shown repeatedly through analysis of large segments of cloned DNA (Beja et al. 2000, Frigaard et al. 2006, Martin-Cuadrado et al. 2008, Moreira et al. 2004) as well as the complete genome (Iverson et al. 2012) to be in the order *Thermoplasmatales*. Phylogenetic analysis based on conserved gene content further refines the position of Group II, showing its closest cultured relative is *Aciduliprofundum boonei* of the Deep Hydrothermal Vent Euryarchaeota taxonomic cluster (Reysenbach & Flores 2008, Reysenbach et al. 2006). This places Group II with the cluster of Euryarchaeota that produce GDGT-1 to -4, including *A. boonei* (Schouten et al. 2008a), *Ferroplasma acidarmanus* (Macalady et al. 2004), and several *Thermoplasma* spp. (e.g., Uda et al. 2001) (**Figure 3**). The gene content of all these sequences points to a heterotrophic or fermentative lifestyle, with respiratory chains indicative of heterotrophic metabolism. The surface-dwelling Group II.a also contains proteorhodopsin, leading to the suggestion that surface strains may be photoheterotrophs (Iverson et al. 2012) and explaining the colocalization of Group II with *Prochlorococcus* in the deep chlorophyll maximum (Ghai et al. 2010).

The ability to produce GDGTs containing cyclopentane rings seems to be common within Thermoplasmatales. Accordingly, Euryarchaeota may be part of the aggregate community that produces GDGTs in the water column or sediments. Other uncultured Euryarchaeota in marine systems include the major sedimentary groups: Marine Benthic Groups D and E as well as SAGMEG (Teske & Sorensen 2008). However, the phylogenetic placement of these groups is not as clear. Whether any of the potential euryarchaeal sources of GDGTs are sufficiently

abundant to affect GDGT distributions in sediments remains unknown. Determining the range of biophysical response of the lipid signatures of all these Euryarchaeota will be important. This requires renewed efforts to bring the Group II planktonic division, as well as sedimentary relatives, into culture. More progress in this area will also come from a better understanding of the genetics of GDGT biosynthesis, eventually enabling a predictive approach to determine which taxa can make tetraethers, diethers, or other novel compounds.

Most paleoceanographic studies based on TEX_{86} ratios assume that GDGT core lipids in marine sediments derive quantitatively from exported biomass of surface-derived, planktonic, ammonia-oxidizing, autotrophic Thaumarchaeota. But as summarized in this review, multiple unanswered questions remain. These questions generally pertain either to the possibility of discovering alternative sources for GDGTs or to our understanding of the physiological basis for the TEX_{86}-temperature relationship; in many cases, the two issues are not easily decoupled. Future studies should focus not only on resolving these questions but also on understanding how the answers affect the regional and temporal applicability of the TEX_{86} paleothermometer.

SUMMARY POINTS

1. Archaea and their GDGT lipids are ubiquitous in the global environment. All known Thaumarchaeota and Crenarchaeota, as well as many taxonomic groups of Euryarchaeota, are able to produce GDGTs and may contribute to lipid signals preserved in marine sediments.
2. The TEX_{86} paleotemperature proxy has opened up new avenues for investigating SST, but the observation that many regions show patterns different from the existing calibration lines argues for a strong need to uncover the underlying causes for these discrepancies. It is essential to understand the patterns observed in the modern ocean if we are to obtain the best possible paleoreconstructions.
3. Planktonic Thaumarchaeota are believed to be the primary nitrifiers in the ocean. Their maximum cell numbers, rates of activity, and biomass carbon isotope signatures are consistent with growth primarily in deep photic and subphotic waters. The exact mechanism by which a primarily chemocline or thermocline-dwelling population can maintain the correlation between TEX_{86} and SST remains unknown.

FUTURE ISSUES

1. How are GDGTs biosynthesized, and what is their direct role in membrane physiology?
2. Do Euryarchaeota contribute GDGTs to sediments, and what is the biophysical relationship between number of cyclopentane rings and temperature, pH, and other variables involved in homeostasis?
3. Do all Thaumarchaeota ammonia oxidizers contain the same suite of GDGTs and exhibit the same range of biophysical regulation?
4. Are any sediment-dwelling Thaumarchaeota capable of making significant quantities of the same lipids in situ, thereby overprinting signals from the water column?
5. How do effects imposed by archaeal community heterogeneity in the water column—including seasonality and/or depth-organized stratification of populations—relate to the observed calibration signal of TEX_{86} SST?

6. How do we best reconcile the different timescales of SST measurements, export production, and sedimentation rate, and what is the integrated export depth of GDGTs from the water column?
7. If terrestrial environments are also sources of GDGTs, can the influence of continental input to ancient marine sediments be reliably detected and excluded?
8. Does the biosynthetic or diagenetic production of alternative structures such as hydroxylated GDGTs or isoprenoid glycerol dialkyl diols affect TEX_{86} ratios and associated paleotemperature estimates (e.g., Liu et al. 2012)?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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