

## 12.14 Biomarker-Based Inferences of Past Climate: The TEX<sub>86</sub> Paleotemperature Proxy

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12.14.1	Introduction	379
12.14.2	History and Systematics	379
12.14.3	Detection and Analysis of GDGTs	381
12.14.4	Ecology of the Thaumarchaeota and Implications for TEX <sub>86</sub>	381
12.14.5	Preservation of GDGT Lipids in Sediments	384
12.14.6	Calibration of TEX <sub>86</sub> to Temperature	384
12.14.6.1	Mesocosm Calibrations	384
12.14.6.2	Surface-Sediment Calibrations	385
12.14.7	Conclusion	390
References		391

### 12.14.1 Introduction

The development of quantitative estimates of past temperature changes is one of the principal goals of paleoclimatology. Such estimates not only reveal the state of the Earth in highly altered climates but also provide fundamental insights into Earth system sensitivity (the amount the planet warms when CO<sub>2</sub> increases, accounting for 'slow' feedbacks; Lunt et al., 2009). Traditionally, paleotemperature estimates have relied on the distribution or chemical composition of hard fossils, such as the oxygen isotopic composition of calcareous foraminifera (Emiliani, 1955; Erez and Luz, 1983). However, during the last 25 years, organic geochemists have developed new techniques based on the distribution of molecular fossils or 'biomarkers,' which in certain sedimentary environments may be preserved where hard fossils are not. These lipid proxies expand our ability to reconstruct temperatures in both geologic time and depositional space.

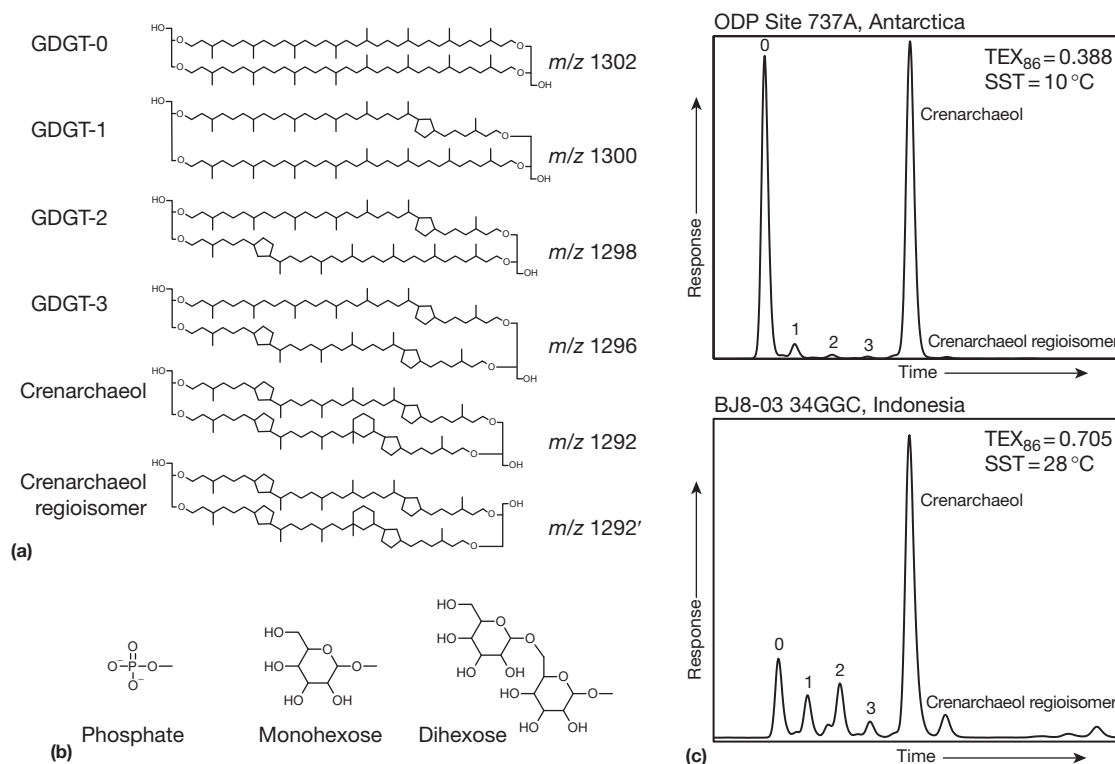
Biomarker temperature reconstructions are based on the principle that microorganisms adjust the rigidity of their cell membranes in response to environmental temperature (e.g., Kita et al., 1973; Nozawa et al., 1974; Ray et al., 1971). By altering the number of double bonds, rings, or branches in their phospho- or glycolipid membranes, microbes raise or lower the melting point of their cell structures and adjust membrane fluidity. Useful lipids for paleotemperature reconstructions must be (1) well preserved and abundant in ancient sediments, (2) relatively easy to isolate and identify, and (3) reasonably specific to an organism such that temperature calibrations can be determined in both controlled experiments and the natural environment. Alkenones and the corresponding U<sub>37</sub><sup>K'</sup> index (Marlowe et al., 1984; Volkman et al., 1980) and a proxy based on the ubiquitous membrane lipids of Thaumarchaeota – TEX<sub>86</sub> (TetraEther index of 86 carbons) – both fulfill these criteria. A detailed review of the U<sub>37</sub><sup>K'</sup> proxy may be found in Chapter 8.15. Here, I focus on discussing TEX<sub>86</sub>; a newer lipid proxy that is increasingly applied in paleoclimate studies, especially in deep geologic time. TEX<sub>86</sub> extends our capabilities to reconstruct paleotemperatures as it (1) allows

evaluation of environmental temperatures higher than U<sub>37</sub><sup>K'</sup>, whose calibration tops out at 28 °C, and (2) allows for temperature estimates far back into geologic history, as TEX<sub>86</sub> derives from lipids of mesophilic Archaea that have been extant for billions of years (Spang et al., 2010; Zhang et al., 2006). So far, TEX<sub>86</sub> has been applied to sediments dating back to the Middle Jurassic period (Jenkyns et al., 2012).

### 12.14.2 History and Systematics

TEX<sub>86</sub> is based on the degree of cyclization of 86-carbon membrane lipids produced by mesophilic marine and lacustrine Thaumarchaeota, such as the ubiquitous ammonia-oxidizing archaeon *Nitrosopumilus maritimus*. Formerly referred to as Group I Crenarchaeota, Thaumarchaeota were recently segregated into a separate phylum on the basis of genetic evidence that suggests they are distinct from their hyperthermophile relatives and deeper-branching within the archaeal phylogeny than expected (Brochier-Armanet et al., 2008; Spang et al., 2010). The Thaumarchaeotal membrane lipids used to calculate TEX<sub>86</sub> are glycerol dialkyl glycerol tetraethers (GDGTs): two head-to-head C<sub>40</sub> isoprenoid chains with a varying number of cyclopentane and cyclohexane rings connected by ether bonds to terminal glycerol groups (Figure 1). Polar headgroups, including monohexose, dihexose, and phosphate moieties (Pitcher et al., 2010; Schouten et al., 2008b; Sturt et al., 2004), are attached to the core GDGT structure when the membrane lipid is intact (Figure 1) but are typically lost during diagenetic alteration. Sedimentary GDGTs with terminal hydroxyl groups are the form most often found in ancient sediments (Figure 1).

Although GDGTs are characteristic lipids of Archaea, they are not specific to Thaumarchaeota and are ubiquitously associated with methanogenic, hyperthermophilic, and mesophilic species. GDGTs were first identified within a culture of the well-studied thermoacidophile *Sulfolobus solfataricus* (previously named *Caldariella acidophila*; de Rosa et al., 1974, 1977), and *S. solfataricus* was observed to produce GDGTs



**Figure 1** (a) Core structures of the isoprenoidal glycerol dialkyl glycerol tetraethers (GDGTs) commonly found in marine sediments and used for the TEX<sub>86</sub> proxy, with mass-to-charge ratios ( $m/z$ ) on the right associated with analysis via HPLC/positive ion APCI. (b) Common polar headgroups of intact GDGTs, which would replace one or both of the terminal hydroxyl groups indicated in (a). (c) High-performance liquid chromatography/mass spectrometry (HPLC/MS) chromatograms of example ‘cold’ and ‘hot’ sedimentary GDGT compositions with corresponding TEX<sub>86</sub> index values and estimated sea-surface temperatures (SSTs) using the TEX<sub>86</sub><sup>H</sup> calibration of Kim et al. (2010). The upper panel shows a trace from Ocean Drilling Program Site 737A in Antarctica and is Pliocene in age (data courtesy of Courtney Warren and Mark Pagani, Yale University). The bottom panel shows a trace from *Baruna Jaya* 8-03 Core 34GGC, a gravity core taken in the Makassar Strait, Indonesia, and is of late Holocene age (Tierney, unpublished data). Numbers correspond to the GDGT structures in (a). GDGTs 1–3 elute with leading shoulders that likely represent isomers of GDGTs 1–3 (Sinninghe Damsté et al., 2002c); these shoulders are more prominent at warmer SSTs (cf. the Indonesian chromatogram).

with more cyclopentyl moieties at higher temperatures (75.89 °C; de Rosa et al., 1980). Gliozzi et al. (1983) subsequently showed that the degree of cyclization within the membrane lipids of *S. solfataricus* altered the transition temperature (melting point) of the lipids, and a similar relationship between cyclization and growth temperature was observed for the thermophile archaeon *Thermoplasma acidophilum* years later (Uda et al., 2001).

Archaea were originally thought to consist only of unusual halophile and thermophile organisms that inhabited extreme and selective environments, such as hot springs and deep-sea hydrothermal vents. Therefore, although it was known that archaeal lipids responded to environmental temperatures, it was of limited use. Furthermore, analysis of GDGTs was difficult (see Section 12.14.3). Skeletal remnants of GDGTs – biphytanes – were commonly found in ancient lacustrine (e.g., the Messel Shale; Chappe et al., 1980) and marine (Brassell et al., 1981) sedimentary environments, but it was not until the early 1990s that mesophilic relatives of extremophile Archaea were discovered to live in marine environments (DeLong, 1992; Fuhrman et al., 1992). Almost a decade later, a comprehensive ribosomal RNA survey of the North Pacific revealed that pelagic Archaea were

commonplace and abundant, potentially comprising up to 20% of the total picoplankton in the global ocean (Karner et al., 2001).

Innovations in high-performance liquid chromatography/mass spectrometry (HPLC/MS) allowed for rapid analysis and elucidation of core GDGT structures without time-intensive chemical pretreatment (Hopmans et al., 2000; see Section 12.14.3) and opened the door for expansive evaluations of GDGTs in marine and sedimentary systems (e.g., Schouten et al., 2000; Sinninghe Damsté et al., 2002c). A unique GDGT containing both cyclopentyl moieties and a cyclohexyl ring not previously found in cultures of hyperthermophiles was discovered and assumed to be specific to marine mesophilic Thaumarchaeota (‘crenarchaeol’; Sinninghe Damsté et al., 2002b). Crenarchaeol was accompanied by a regioisomer (Sinninghe Damsté et al., 2002b,c), and both compounds appeared abundant in tropical marine environments (Sinninghe Damsté et al., 2002c). Sinninghe Damsté et al. (2002b) theorized that the cyclohexyl ring in crenarchaeol represented a unique adaptation of the mesophilic Archaea to cooler marine temperatures, in so far that its presence helped increase membrane volume and stability. However, crenarchaeol has since been found in hot springs

(Pearson et al., 2004) and cultures of the thermophile *Nitrosocaldus yellowstonii* (de la Torre et al., 2008) and the moderate thermophile *Nitrososphaera gargensis* (Pitcher et al., 2010). The functional role of the cyclohexane ring in crenarchaeol remains a matter of debate (de la Torre et al., 2008; Pearson et al., 2004; Pitcher et al., 2009, 2010; Zhang et al., 2006).

Stefan Schouten and colleagues at the Royal Netherlands Institute for Sea Research (NIOZ) noted a relationship between the degree of cyclization in marine surface sediments and overlying sea-surface temperature (SST) (Schouten et al., 2002). The acyclic GDGT-0 dominated sediments from polar regions, whereas GDGTs-2 and -3, crenarchaeol, and its regioisomer were more abundant in tropical sediments (Figure 1). Schouten et al. (2002) used this qualitative observation to define the TetraEther index of 86 carbons (TEX<sub>86</sub>): a ratio expressing the degree of cyclization that best correlated with SST (Schouten et al., 2002; see Figure 1 for structures; 'cren' refers to the crenarchaeol regioisomer):

$$\text{TEX}_{86} = \frac{[\text{GDGT-2}] + [\text{GDGT-3}] + [\text{cren}']}{[\text{GDGT-1}] + [\text{GDGT-2}] + [\text{GDGT-3}] + [\text{cren}']} \quad [1]$$

Calibration of TEX<sub>86</sub> to temperature resulted in a linear expression:

$$\text{TEX}_{86} = 0.015 \times T + 0.28 \quad (r^2 = 0.92, n = 43) \quad [2]$$

The first TEX<sub>86</sub>-based paleoclimate reconstruction targeted the warmth of the Cretaceous greenhouse climates (Schouten et al., 2003), taking advantage of the fact that high concentrations of GDGTs were previously detected in black shales associated with ocean anoxic events (Kuypers et al., 2001). A few years later, TEX<sub>86</sub> was first applied to Late Quaternary sediments in the Arabian Sea (Huguet et al., 2006c) and has been applied to marine sequences spanning a variety of geologic timescales since then. TEX<sub>86</sub> has also been used to infer past temperature change in select tropical freshwater lakes where the proxy appears applicable (Powers et al., 2005; Tierney et al., 2008, 2010a).

### 12.14.3 Detection and Analysis of GDGTs

Due to their high molecular weight and polarity, both intact (i.e., with polar headgroups) and core GDGTs are not easily resolved by gas chromatography. Early investigations of GDGT lipids relied on acid hydrolysis (for intact polar GDGTs), careful chromatographic separation, and chemical degradation in order to indirectly analyze these lipids (e.g., Chappe et al., 1980; de Rosa et al., 1980; Hoefs et al., 1997). Such procedures usually involve thin-layer chromatography or silica gel separation to isolate core GDGTs, followed by treatment with 57% hydroiodic acid (HI) to cleave the ether bonds connecting the C<sub>40</sub> biphytanyl chains to the glycerol units. The resulting alkyl iodides are then reduced to C<sub>40</sub> biphytanes by treatment with LiAlH<sub>4</sub> to be analyzed by gas chromatography. This approach has several limitations: (1) because the GDGTs are split into biphytanes, it is not possible to identify the core structures of each GDGT from which they derive, and (2) HI cleavage and LiAlH<sub>4</sub> reduction is a laborious process and, as a result, prohibitive for highly resolved paleotemperature reconstructions.

The 1980s and 1990s witnessed an expansion in the sophistication, automation, and affordability of commercially available high-performance liquid chromatography/mass-spectrometry systems (Snyder et al., 2009) that enabled rapid detection of high molecular weight, polar compounds. In 2000, Ellen Hopmans and colleagues at the Royal Netherlands Institute for Sea Research (NIOZ) developed a new method for analysis of core GDGT structure via normal-phase HPLC and atmospheric pressure chemical ionization (APCI) mass spectrometry (Hopmans et al., 2000), involving only a simple purification of total lipid extracts prior to HPLC analysis (e.g., elution over aluminum oxide solid phase with dichloromethane/methanol (1:1); Schouten et al., 2002). Further methodological improvements over the last decade, including the use of single ion monitoring (SIM; Huguet et al., 2006b; Schouten et al., 2007b), enhanced the precision of TEX<sub>86</sub> measurements to 0.004 TEX<sub>86</sub> units or better, which corresponds to about 0.3 °C (depending on the temperature calibration used). For specific HPLC/APCI-MS operating conditions, see Schouten et al. (2007b). Typical HPLC/APCI-MS chromatograms of marine sedimentary GDGTs are shown in Figure 1.

The TEX<sub>86</sub> index is a ratio and thus does not require absolute quantitation to calculate, but quantitation of total GDGT abundance in sediments may be desirable as an indicator of the production and/or preservation of archaeal biomass. A synthetic C<sub>46</sub> GDGT standard (Patwardhan and Thompson, 1999) can be used to achieve quantitation, once the relative response factor of the higher molecular weight GDGTs versus the standard is accounted for (Huguet et al., 2006b). Since the C<sub>46</sub> standard behaves similar to GDGTs chromatographically and chemically, it can be added to the sediment prior to extraction to correct for losses during the extraction and preparatory procedures.

### 12.14.4 Ecology of the Thaumarchaeota and Implications for TEX<sub>86</sub>

The discovery that marine Thaumarchaeota represented a substantial picoplankton component (Karner et al., 2001) suggested that mesophilic Archaea might play an important role in global ocean nutrient cycling, although metabolic characteristics were not precisely known at the time. Thaumarchaeota had yet to be isolated, so early inferences regarding their ecology were limited to enrichment experiments of ocean water, the study of characteristic archaeal lipid biomarkers (viz., GDGTs), and genetic surveys. An rRNA survey of archaeal populations from the Santa Barbara basin (Massana et al., 1997) determined that Euryarchaeota dominated surface waters, whereas Thaumarchaeota resided below the photic zone, suggesting distinctions in metabolic processes and physiology. Several research groups observed that the stable carbon isotopic signature (<sup>13</sup>C) of biphytane lipids was characteristically enriched in <sup>13</sup>C relative to heterotrophic lipids, implying autotrophic uptake of bicarbonate (Hoefs et al., 1997; Kuypers et al., 2001; Pearson et al., 2001). Radiocarbon analyses of biphytanes also suggested that Thaumarchaeota were utilizing old dissolved inorganic carbon (DIC) from below the photic zone rather than younger, newly fixed organic carbon (Pearson et al., 2001). A subsequent enrichment

and labeling experiment confirmed these suspicions, demonstrating that Thaumarchaeota incorporate bicarbonate (Wuchter et al., 2003).

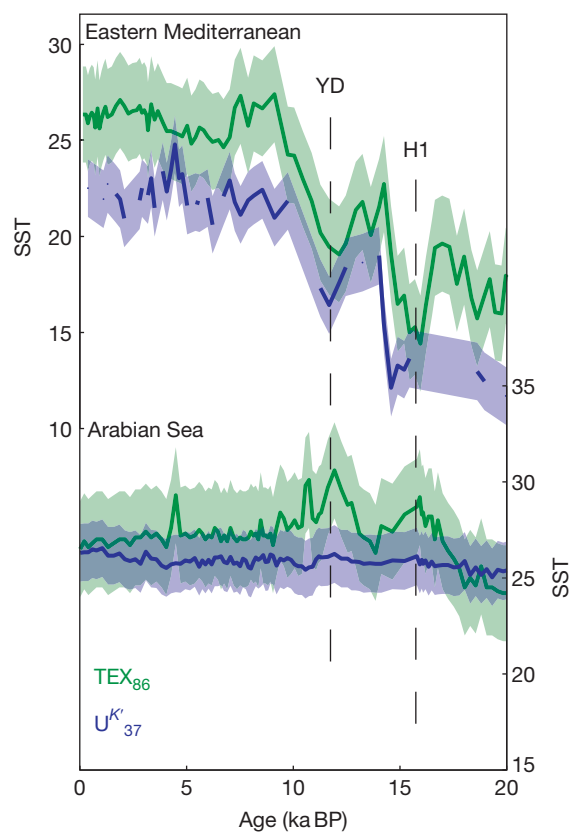
Technical innovations in the field of metagenomics – direct cloning of genetic material from environmental samples (Handelsman, 2004) – aided the study of Thaumarchaeota, allowing detection of important genetic traits in the absence of laboratory cultivation. As part of a shotgun sequencing study of the Sargasso Sea, Venter et al. (2004) discovered an ammonium monooxygenase gene on an archaeal scaffold, providing the first genetic evidence that marine Thaumarchaeota were engaged in ammonium oxidation. In addition, a study of rRNA in soils where mesophilic Archaea are abundant (Ochsenreiter et al., 2003) yielded detection of *amoA* genes on archaeal fragments (Schleper et al., 2005). This information, in conjunction with lipid and experimental evidence for autotrophic assimilation of bicarbonate, suggested that some species of Thaumarchaeota were chemolithoautotrophic ammonium oxidizers (nitrifiers). Könneke et al. (2005) isolated a marine archaeon and directly observed chemolithoautotrophic nitrification by marine Thaumarchaeota, confirming this suspicion. We now recognize that Archaea are intimately involved in marine nitrogen cycling (Francis et al., 2005; Könneke et al., 2005).

Nitrifying capabilities fit nicely with accumulated evidence from water-column surveys of both GDGT lipids and archaeal genetic material suggesting that Thaumarchaeota preferentially reside in the mesopelagic region below the photic zone (Herndl et al., 2005; Huguet et al., 2007; Ingalls et al., 2006; Karner et al., 2001; Massana et al., 1997; Turich et al., 2007; Wakeham et al., 2003; Wuchter et al., 2005, 2006). However, these observations present a conceptual problem for the TEX<sub>86</sub> proxy: empirically, TEX<sub>86</sub> correlates best to SST or temperatures between 0 and 100 m (Kim et al., 2008, 2010), yet Thaumarchaeota typically reside deeper within the water column. Differences in the export pathways of surface and deeper-water GDGTs provide a partial explanation. In a study of GDGTs in the Black Sea, Wakeham et al. (2003) observed that in spite of active anaerobic oxidation of methane (AOM) by Archaea in the water column and sediments, there was no distinctive signal for AOM in GDGT lipids preserved in either the sediments or deep sediment traps (Wakeham et al., 2003). Rather, deep-water and sediment GDGT compositions were typical of the upper water column. Archaeal cells are extremely small (~1 μm) and do not effectively sink on their own. Thus, Wakeham et al. (2003) speculated that grazers play an important role in exporting GDGTs from the surface ocean to the sediments as fecal pellets, whereas the absence of a grazing community in deep waters prohibits the preservation and export of archaeal GDGTs.

An alternative explanation to the ‘surface export hypothesis’ posits that TEX<sub>86</sub> always records subsurface temperatures, and the observed empirical statistical correlation to SST is merely a reflection of the fact that temperatures at ~100 m are highly and significantly correlated with SSTs, at least spatially (Huber, 2010). Therefore, it is nearly impossible to statistically determine whether surface or subsurface temperatures are better correlated with TEX<sub>86</sub> in the context of a spatial calibration. It is also possible that the higher fidelity of SST measurements (which consist of many in situ and satellite estimates) over

subsurface temperature measurements (which consist of sporadic in situ data) may result in artificially lower correlations between TEX<sub>86</sub> and deeper-water temperatures. According to the ‘correlation hypothesis,’ cases in which TEX<sub>86</sub> appears to record SST variability through time merely reflect the fact that subsurface temperatures vary in concert with SSTs.

Some paleoclimate data seem to affirm that TEX<sub>86</sub> records SST, whereas others suggest that TEX<sub>86</sub> reflects the subsurface ocean as would be expected from the ecology of Thaumarchaeota. Castañeda et al. (2010) reconstructed SSTs with TEX<sub>86</sub> and U<sub>37</sub><sup>K'</sup> in the eastern Mediterranean Sea for the last 25 000 years and found generally good agreement between the proxies; both recorded similar variability and magnitude of change, though with a different mean value (Figure 2). In this case, TEX<sub>86</sub> arguably reflects SSTs, or else, subsurface temperatures in the Mediterranean behave similarly to SSTs on the time-scales captured by the data. In contrast, Huguet et al. (2006c) found poor agreement between TEX<sub>86</sub>- and U<sub>37</sub><sup>K'</sup>-inferred SSTs over a similar time frame in the Arabian Sea. TEX<sub>86</sub> data



**Figure 2** TEX<sub>86</sub> and U<sub>37</sub><sup>K'</sup> SST reconstructions from the eastern Mediterranean (Castañeda et al., 2010) and the Arabian Sea (Huguet et al., 2006c) suggest that TEX<sub>86</sub> can feasibly reflect either surface or subsurface temperatures. TEX<sub>86</sub> and U<sub>37</sub><sup>K'</sup> are calibrated to SST with the Kim et al. (2010) TEX<sub>86</sub><sup>H</sup> calibration and the Müller et al. (1998) calibrations, respectively, and are plotted with the 1σ calibration error envelope. In the eastern Mediterranean, TEX<sub>86</sub> and U<sub>37</sub><sup>K'</sup> show generally similar variability with an offset in the mean. In the Arabian Sea, TEX<sub>86</sub> and U<sub>37</sub><sup>K'</sup> record very different signatures; here, TEX<sub>86</sub> conceivably reflects subsurface temperatures (see main text). YD and H1 mark the Younger Dryas and Heinrich Event 1, respectively.



recorded large warmings during North Atlantic stadial events the Younger Dryas and Heinrich Event 1, whereas U<sub>37</sub><sup>K'</sup> registered no response (Figure 2). As these events are thought to have caused cooling throughout the Northern Hemisphere (e.g., Dahl et al., 2005), the TEX<sub>86</sub> SST estimates of nearly 30 °C are unrealistic. However, if we assume Thaumarchaeota live closer to the thermocline, then relatively warmer TEX<sub>86</sub> temperatures (though not the absolute values) would agree with independent proxy evidence that Arabian Sea upwelling was reduced (Altabet et al., 2002; Schulz et al., 1998), deepening the thermocline and raising subsurface temperatures. As is the case with the Arabian Sea, other reports that TEX<sub>86</sub> reflects subsurface temperatures come from areas with dynamic oceanography, including the Guinea margin (Lopes Dos Santos et al., 2010) and the Peru margin (Chazen, 2011). In such locations, temporal variability in subsurface temperature might be expected to be substantially different from that of SSTs, perhaps lending credence to the 'correlation hypothesis.'

Because GDGTs are not specific to Thaumarchaeota, changes in archaeal communities can impact sedimentary GDGT distributions and influence TEX<sub>86</sub> independent of environmental temperature. For example, in areas where archaea performing AOM are dominant TEX<sub>86</sub> is not related to SST due to the contribution of GDGTs 0–3 from AOM archaea (Blumenberg et al., 2004; Zhang et al., 2011). Similarly, in the Red Sea, TEX<sub>86</sub> values deviate significantly from the open-ocean relationship to SST (Trommer et al., 2009, and see discussion in Section 12.14.6), likely reflecting the unique archaeal populations in this semienclosed basin. Novel species of Thaumarchaeota have been detected in the deep-water brines of the northern Red Sea (Eder et al., 2002), and genetic evaluations of Archaea in the Gulf of Aqaba found evidence of distinct lineages of Thaumarchaeota below the thermocline (Ionescu et al., 2009). Pyrosequencing of waters overlying deep brine pools (the Atlantis II Deep and the Discovery Deep) within the central Red Sea determined that Halobacteriales (Euryarchaeota) dominated surface waters and Desulfurococcales (Crenarchaeota) dominated deeper waters (Qian et al., 2010). Desulfurococcales produce both acyclic and cyclic GDGTs (Knappy et al., 2011) and could conceivably influence the sedimentary TEX<sub>86</sub> signatures in the Red Sea.

Applications of TEX<sub>86</sub> also generally assume that Thaumarchaeota, rather than Euryarchaeota, are the dominant archaeal representatives in the surface ocean. But Euryarchaeota are known to populate the surface ocean, and cultures of extremophilic relatives produce GDGTs 0–3 (Gattinger et al., 2002; Schouten et al., 2008a). In a survey of water-column GDGT lipids, Turich et al. (2007) suggested that in fact, surface ocean GDGTs are more likely derived from Euryarchaeota and sedimentary GDGT archives must reflect contributions from both epipelagic Euryarchaeota and mesopelagic Thaumarchaeota. Consequently, any change in the relative contribution of each archaeal group to the sedimentary GDGT signature could distort TEX<sub>86</sub>-based temperature estimates. Schouten et al. (2008c) disputed these findings, noting that a study of archaeal populations in the North Sea showed that GDGT fluxes matched the seasonal productivity of Thaumarchaeota and not Euryarchaeota (Wuchter, 2006). The degree to which GDGTs derived from Euryarchaeota influence TEX<sub>86</sub> remains unresolved.

Seasonal production of GDGTs by marine Archaea could also bias the sedimentary TEX<sub>86</sub> signature. Thaumarchaeota show seasonal cycles in abundance (Galand et al., 2010; Hollibaugh et al., 2010; Massana et al., 1997; Pitcher et al., 2011) as do their lipids (Pitcher et al., 2011; Wuchter et al., 2006). The timing of peak abundance varies from environment to environment, possibly related to seasonal changes in ammonium availability and/or competition with phytoplankton. Thaumarchaeota are most productive in the wintertime in the North Sea, Santa Barbara Basin, and offshore Spain (Galand et al., 2010; Massana et al., 1997; Pitcher et al., 2011), in the summertime offshore the southeastern United States (Hollibaugh et al., 2010), and during the southwest monsoon upwelling season in the Arabian Sea (Wuchter et al., 2006). Whether seasonal production biases the sedimentary TEX<sub>86</sub> signal is an open question; Rueda et al. (2009) observed that sedimentary TEX<sub>86</sub> reflected mean annual SST variability in the North Sea, and Wuchter et al. (2006) note that their deepest sediment trap in the Arabian Sea records mean annual SST, reflecting a homogenization of the seasonal cycle. On the other hand, many TEX<sub>86</sub>-based paleoclimatic studies invoke a seasonal bias of the TEX<sub>86</sub> proxy to explain either absolute temperature values or observed trends (e.g., Castañeda et al., 2010; Huguet et al., 2006c; Shevenell et al., 2011; Sluijs et al., 2011). This is often a post hoc assumption, designed to address a mismatch between calibrated TEX<sub>86</sub> temperatures (which may have large residual biases in some areas of the global ocean; see the succeeding text) and modern-day SSTs rather than grounded in empirical data. Further studies that focus on export pathways of marine GDGTs will be key to establishing how seasonal production of GDGTs imprints the sedimentary record.

Thaumarchaeota and their GDGTs are also abundant in soils (Leininger et al., 2006; Ochsenreiter et al., 2003; Weijers et al., 2006), albeit at lower concentrations relative to marine sediments (Weijers et al., 2006). This raises the possibility that soil GDGTs influence application of the TEX<sub>86</sub> proxy where terrestrial organic matter input is important. The branched and isoprenoidal tetraether (BIT) index – a ratio between soil-derived branched GDGTs and crenarchaeol that is applied as a measure of terrestrial organic matter input to nearshore ocean sediments (Hopmans et al., 2004) – is often used as a guide to determine if the marine TEX<sub>86</sub> signature is influenced by soil contributions. Weijers et al. (2006) proposed that sediments with BIT > 0.3 would have substantially biased TEX<sub>86</sub> temperature estimates, based on a mixing model study of GDGTs in the Niger River fan. However, BIT is a nonlinear indicator of soil organic matter that is highly sensitive to the absolute concentrations of crenarchaeol and branched GDGTs (Smith et al., 2012), and the critical BIT value will also depend on the concentration of GDGTs in respective soil types. For example, Richey et al. (2011) found no impact of soil GDGTs on the TEX<sub>86</sub> proxy in the Gulf of Mexico, in spite of large contributions of terrestrial organic matter from the Mississippi River and BIT values that exceeded 0.3. The BIT cutoff point will widely vary from basin to basin, and there is no universally applicable value, although it is often assumed that there is.

Thaumarchaeota and GDGTs are also abundant in freshwater lakes (Llirós et al., 2010; Powers et al., 2004, 2005; Schouten et al., 2012; Tierney et al., 2008, 2010a,b) allowing for limited applications of TEX<sub>86</sub> in terrestrial settings, namely, in the large tropical lakes of East Africa (Powers et al., 2005;

Tierney et al., 2008, 2010a). In these lakes, Thaumarchaeota reside in the upper mixed layer (<100 m) near the nutricline, as is expected given their ammonium-oxidizing ecology (Llirós et al., 2010). Schouten et al. (2012) showed that GDGT concentrations also peak near the nutricline/oxycline in Lake Tanganyika (~80 m) and that sedimentary TEX<sub>86</sub> matched TEX<sub>86</sub> measured in suspended particulate matter from the upper water column (0–100 m), similar to what has been observed in the ocean. However, application of TEX<sub>86</sub> in these large lakes necessitates separate calibrations (Tierney et al., 2010a; Powers et al., 2010, and see the succeeding text), suggesting that lacustrine and marine Thaumarchaeota might respond differently to temperature. In most small lake environments, soil GDGTs and the prevalence of methanogenic archaea appear to complicate TEX<sub>86</sub> (Blaga et al., 2009). The effects of these influences do not seem systematic. For example, Lago Zirahuen, a lake used in the Powers et al. (2010) and Tierney et al. (2010a) calibrations, has a BIT index of 0.53 and a ratio of GDGT-0 to crenarchaeol (used as a guideline for the presence of methanogenic archaea, as these archaea make substantial amounts of GDGT-0) of 0.89, and its TEX<sub>86</sub> value predicts a reasonable lake surface temperature (LST) (Powers et al., 2010). In contrast, Lake Oku has a BIT of 0.53 and a lower GDGT-0/crenarchaeol ratio (0.58), but its TEX<sub>86</sub> value predicts a LST that is 10 °C too cold (Powers et al., 2010). More studies of lacustrine Thaumarchaeota are needed to identify what contributes to such large residual errors of the TEX<sub>86</sub> proxy in certain lakes.

### 12.14.5 Preservation of GDGT Lipids in Sediments

As with any biomarker-based proxy, determining the impacts of early diagenesis and catagenesis is critical. A study of GDGTs and TEX<sub>86</sub> in decapods indicated that TEX<sub>86</sub> was not altered during the passage of the lipids through the guts of grazers (Huguet et al., 2006a), and surveys of both oxic and anoxic sediments show that TEX<sub>86</sub> is stable and not significantly impacted by oxic degradation within analytical error (Huguet et al., 2009; Kim et al., 2009; Schouten et al., 2004; Sinninghe Damsté et al., 2002a). GDGTs do, however, appear to degrade faster than both alkenones and allochthonous soil branched tetraethers. A study of the BIT index across an oxidation front on the Madeira Abyssal Plain showed that BIT substantially increased in oxic sediments due to selective degradation of crenarchaeol (Huguet et al., 2008). Radiocarbon analysis of alkenones and crenarchaeol in continental margin sediments showed that crenarchaeol contained higher <sup>14</sup>C content than co-occurring alkenones, suggesting that GDGTs degrade more readily in oxic waters (Mollenhauer et al., 2008). Thus, while the relative concentrations of GDGTs appear unaffected by diagenetic conditions – preserving TEX<sub>86</sub> values – absolute concentrations of GDGTs will reflect the degree of exposure to oxygenated environments.

A maturation experiment (hydrous pyrolysis of a sulfur-rich sediment containing GDGTs) indicated that GDGTs thermally degrade between 240 and 300 °C and are not detected at temperatures higher than 300 °C (Schouten et al., 2004). As GDGTs degrade (>240 °C), TEX<sub>86</sub> values decline indicating preferential destruction of GDGTs with more cyclopentane

rings (Schouten et al., 2004). Based on these results, Schouten et al. (2004) suggested that TEX<sub>86</sub> is generally not applicable in more thermally mature sediments (e.g., those with high amounts of 22S hopanes; 17 $\alpha$ , 21 $\beta$  (H)-hopane 22S/(22S + 22R) ratios >0.1).

### 12.14.6 Calibration of TEX<sub>86</sub> to Temperature

There are two ways to calibrate TEX<sub>86</sub> to SST: an experimental approach involving culturing Thaumarchaeota under a variety of controlled conditions and an empirical approach establishing relationships between core-top sediments and overlying SST. As with other paleoceanographic temperature proxies, the empirical method is favored because it accounts for the natural environmental conditions (seasonality of production, transport, degradation, and early diagnosis) that influence the expression of sedimentary GDGTs. However, potential competing influences on TEX<sub>86</sub> ratios (e.g., nutrients and light) are not easily isolated via the empirical approach and ideally need to be rigorously evaluated under controlled conditions.

#### 12.14.6.1 Mesocosm Calibrations

To date, only mesocosm experiments with natural (heterogeneous) mesophilic archaeal populations – as opposed to species-specific culture calibrations – have been performed. Wuchter et al. (2004) collected seawater from the North Sea and conducted two incubation series tests using water that had been heated to 27 °C (series I) and cooled to 13 °C (series II) from the initial temperature of 15 °C, respectively. In the both series, tanks were incubated from 5 to 35 °C at roughly 5 °C intervals. Sensitivity to changing salinity was tested by comparing separate incubations at 27‰ versus 35‰, and no significant offset in TEX<sub>86</sub> was observed. In incubation series I, Wuchter et al. (2004) observed little change in TEX<sub>86</sub> between 5 and 10 °C, but a monotonic linear increase in TEX<sub>86</sub> from 10 to 25 °C. In the second incubation series, TEX<sub>86</sub> increased between 15 and 22 °C but then plateaued from 22 to 25 °C. Notably, the slope of TEX<sub>86</sub>-temperature line in the first incubation experiment was identical to the slope of the Schouten et al. (2002) core-top calibration but differed significantly in its y-intercept. Wuchter et al. (2004) noted that this offset could be partially explained by the relative absence of the crenarchaeol isomer in the GDGTs analyzed from the mesocosm. Whereas the crenarchaeol regioisomer typically accounts for approximately 10% of total amount of GDGT present in core-top samples from the tropical oceans, it only accounted for approximately 0.6% in the mesocosm incubated between 25 and 30 °C (Wuchter et al., 2004).

Schouten et al. (2007a) incubated water collected from the tropical Indian Ocean with the intended goal of calibrating TEX<sub>86</sub> to SSTs encountered in ancient greenhouse climates (SSTs > 30 °C). As in the Wuchter et al. (2004) experiments, a linear increase in TEX<sub>86</sub> values was observed between temperatures of 25 and 40 °C, but lower abundances of the crenarchaeol regioisomer were expressed. Both the Wuchter et al. (2004) and Schouten et al. (2007a) studies observed maximum GDGT concentrations at 25 °C, potentially indicating the optimal growth temperature for marine mesophilic

archaea. However, Schouten et al. (2007a) also observed high concentrations of GDGTs between 34 and 36 °C. Interestingly, analysis of archaeal rDNA fragments from the mesocosm at 34–36 °C suggested that the species present differed from the predominant species at 25 °C. These results indicate that a variety of Thaumarchaeota (and possibly Euryarchaeota, though they were not explicitly characterized) were present in the mesocosms and that the community itself changed in response to temperature. In spite of (or possibly because) of this change in community structure, the relationship between TEX<sub>86</sub> and incubation temperature maintained a similar linear slope and y-intercept as was found in the Wuchter et al. (2004) study, indicating a consistent relationship between membrane cyclization and temperature.

Given the importance of the crenarchaeol regioisomer in the temperature calibration of TEX<sub>86</sub>, its relative absence in culture precludes use of these results in paleoclimatic reconstruction. The reason for the lack of the isomer in cultures is not understood. In a subsequent study of the membrane lipid composition of the marine archaeon *Nitrosopumilus maritimus*, the regioisomer was not detected, suggesting that another species is responsible for its production in natural marine environments (Schouten et al., 2008b). Radiocarbon measurements of individual GDGTs from the Bermuda Rise and the Santa Monica basin show that the regioisomer was significantly depleted in radiocarbon relative to the other GDGTs, supporting a novel microbial origin or perhaps creation from the early diagenetic isomerization of crenarchaeol (Shah et al., 2008). Given that questions surround its origin, an argument could be made for excluding the regioisomer from the TEX<sub>86</sub> index, although as discussed in Wuchter et al. (2004), this still does not rectify discrepancies between the mesocosm and core-top calibrations. Furthermore, inclusion of the regioisomer appears to improve TEX<sub>86</sub>-based prediction of SST using core-top sediments, in part because it maintains a steeper calibration slope (Shah et al., 2008). The manner in which the regioisomer's separate origin impacts the accuracy of TEX<sub>86</sub>-based temperatures over time and space has not been resolved.

### 12.14.6.2 Surface-Sediment Calibrations

Global surface-sediment TEX<sub>86</sub> calibrations arguably incorporate the full range of variation attributable to temperature and nontemperature effects and capture the widest uncertainty

range possible. Calibration uncertainty is then taken as an honest reflection of environmental uncertainty on geologic timescales – calibration-in-space is substituted for calibration-in-time. While this is a necessary compromise, the implicit assumption is that SST variability is ergodic (exhibits the same behavior in time as in space). This is not necessarily the case and can result in either overestimation or underestimation of ancient temperatures. For example, temperature reconstructions during greenhouse climates of the early Eocene or middle Cretaceous rely on extrapolations of the modern temperature calibrations beyond 30 °C, and thus, modern calibration uncertainty necessarily underestimates the uncertainty of such inferred temperatures. Conversely, uncertainty related to SST reconstructions from a single tropical ocean locality for the late Holocene would not be associated with uncertainties related to seasonality of production in the high latitudes, and thus, assumption of the global calibration uncertainty could be an overestimation. There are also ecological and evolutionary sources of uncertainty that may not be well represented within modern sediment calibrations; as discussed previously, mesocosm experiments suggest that the response to GDGT distribution to temperature is a community effect dictated by the character of archaea populations. Ignorance of how ecologic frameworks have varied over time and space thus compromises our confidence in the accuracy of TEX<sub>86</sub> temperatures, particularly in ancient worlds for which we have no modern analog.

Increasing application of the TEX<sub>86</sub> proxy and the resulting controversy of some of the results (e.g., high-latitude temperatures of 30 °C during the Paleocene–Eocene thermal maximum (PETM); Sluijs et al., 2011) have driven substantial discussion and analysis of the TEX<sub>86</sub> calibration, including testing the fidelity of the TEX<sub>86</sub> index itself as the best expression of GDGT cyclization and the proposal of nonlinear calibration models.

Table 1 lists all of the proposed TEX<sub>86</sub> calibrations to date and their source references. Some TEX<sub>86</sub> calibrations (2, 3, and 5) treat TEX<sub>86</sub> as the dependent variable. While this is etiologically correct in the sense that TEX<sub>86</sub> responds to SST, prediction of SST from TEX<sub>86</sub> is a separate statistical relationship and one that requires an assessment of uncertainty – in terms of °C – of the predictive approach. Simple inversion to solve for SST will not give an accurate assessment of this uncertainty. Thus, subsequent calibrations (4 and 6–10) chose to treat TEX<sub>86</sub> as the independent variable in order to gain an estimate of predictive uncertainty.

**Table 1** Sediment core-top TEX<sub>86</sub> calibrations

Range	Eqn #	Equation	n	r <sup>2</sup>	RMSE	References
0–30	[2]	TEX <sub>86</sub> = 0.015 × T + 0.28	43	0.92	N/A	Schouten et al. (2002)
30	[3]	TEX <sub>86</sub> = 0.027 × T + 0.016	24	0.78	N/A	Schouten et al. (2003)
5–30	[4]	T = -10.78 + 56.2 × TEX <sub>86</sub>	223	0.94	1.7	Kim et al. (2008)
25–28 <sup>a</sup>	[5]	TEX <sub>86</sub> = 0.035 × T - 0.09	11	0.90	N/A	Trommer et al. (2009)
-3 to 30	[6]	T = 50.475 - 16.332 × (1/TEX <sub>86</sub> )	287	0.82	3.7	Liu et al. (2009)
-3 to 30	[7]	T = 49.9 + 67.5 × (TEX <sub>86</sub> <sup>L</sup> )	396	0.86	4.0	Kim et al. (2010)
5–30	[8]	T = 38.6 + 68.4 × (TEX <sub>86</sub> <sup>H</sup> )	396	0.86	2.5	Kim et al. (2010)
4–30 <sup>b</sup>	[9]	T = -14.0 + 55.2 × (TEX <sub>86</sub> )	12	0.86	3.6	Powers et al. (2010)
10–30 <sup>b</sup>	[10]	T = 3.50 + 38.9 × (TEX <sub>86</sub> )	13	0.92	2.1	Tierney et al. (2010a)

<sup>a</sup>Red Sea calibration.

<sup>b</sup>Lake calibrations.

Equation [3], with a steeper slope than the original calibration of Schouten et al. (2002, eqn [2]), is a subset of the Schouten et al. (2002) core-top dataset and was formulated for estimating the warmer temperatures of the Cretaceous (Schouten et al., 2003). A steeper slope results in a smaller SST change at the high end of the TEX<sub>86</sub> scale and was largely a post hoc modification made to satisfy assumptions regarding maximum possible temperatures and produce better agreement with temperature estimates from pristine foraminifera (Schouten et al., 2003). In contrast, upon expanding the original Schouten et al. (2002) dataset to include 287 core tops, Kim et al. (2008) concluded that there was no statistical basis for assuming a higher sensitivity of the TEX<sub>86</sub> proxy at the warm end of the calibration (20–30 °C). Kim et al. (2008) also noted a large degree of scatter in TEX<sub>86</sub> at temperatures below 5 °C and suggested that TEX<sub>86</sub> was less reliable in this temperature range. The resulting linear calibration (eqn [4], Table 1) was limited to 5–30 °C (thereby excluding all samples from the Arctic and Southern Ocean), excluded core-top data with >±1 standardized residuals, and excluded all data from the Red Sea due to abnormally high TEX<sub>86</sub> values relative to open-ocean sites of comparable SST. A subsequent surface-sediment transect study of TEX<sub>86</sub> in the Red Sea found that sediments from the northern end of the basin significantly deviated from the global calibration but nonetheless displayed a linear correlation with SST (Trommer et al., 2009). In contrast, the data from the southern end of the Red Sea did not track local SST but appeared to define a mixing line between the TEX<sub>86</sub> signals typical of the Gulf of Aden and the northern Red Sea (Trommer et al., 2009). From this observation, Trommer et al. (2009) hypothesized that the semienclosed environment of the northern Red Sea harbored distinct archaeal populations – and therefore a different TEX<sub>86</sub> response to temperature – from the open-ocean populations in the Gulf of Aden, a hypothesis supported by genetic evidence for diverse archaea (see discussion in Section 12.14.4). Trommer et al. (2009) argued that TEX<sub>86</sub> still responds to SSTs in the northern Red Sea and devised a local calibration (eqn [5], Table 1), but the accuracy of this regional calibration necessarily assumes that the unique Red Sea archaeal populations have remained similar through time.

Liu et al. (2009) proposed a nonlinear transformation of TEX<sub>86</sub> as part of a global, multiproxy reconstruction of temperature change across the Eocene/Oligocene boundary, noting that the Kim et al. (2008) linear calibration produced unreasonably warm temperatures during the middle Cretaceous (Schouten et al., 2003) and in the Eocene polar oceans (Sluijs et al., 2006). Given the potential for ecological and other non-temperature effects, and the inevitable uncertainty as to how these effects translate from region to region over geologic time, Liu et al. (2009) argued that reduction of calibration uncertainty through restriction of any available data provided an unrealistic perspective of the real temperature uncertainty. Their calibration, based on the reciprocal of TEX<sub>86</sub> (eqn [6]), used the same 287 core tops as the Kim et al. (2008) study but did not exclude any outlying data (including data from the Red Sea). Like the Schouten et al. (2003) calibration, this functional form implied an enhanced sensitivity of TEX<sub>86</sub> at higher SSTs (small changes in warm SSTs cause large changes in TEX<sub>86</sub>).

Continuing with the nonlinear calibration approach, Kim et al. (2010) presented a new set of TEX<sub>86</sub> indices based on the

log (base 10) transformation of TEX<sub>86</sub>. Kim et al. (2010) also revisited the formulation of the TEX<sub>86</sub> index itself, using an iterative method to identify the best index ratio to relate to SST given an expanded set of 396 core tops. When all of the data were considered (except for data from the Red Sea), the most statistically robust index was

$$\log\left(\frac{[\text{GDGT-2}]}{[\text{GDGT-1}] + [\text{GDGT-2}] + [\text{GDGT-3}]}\right)$$

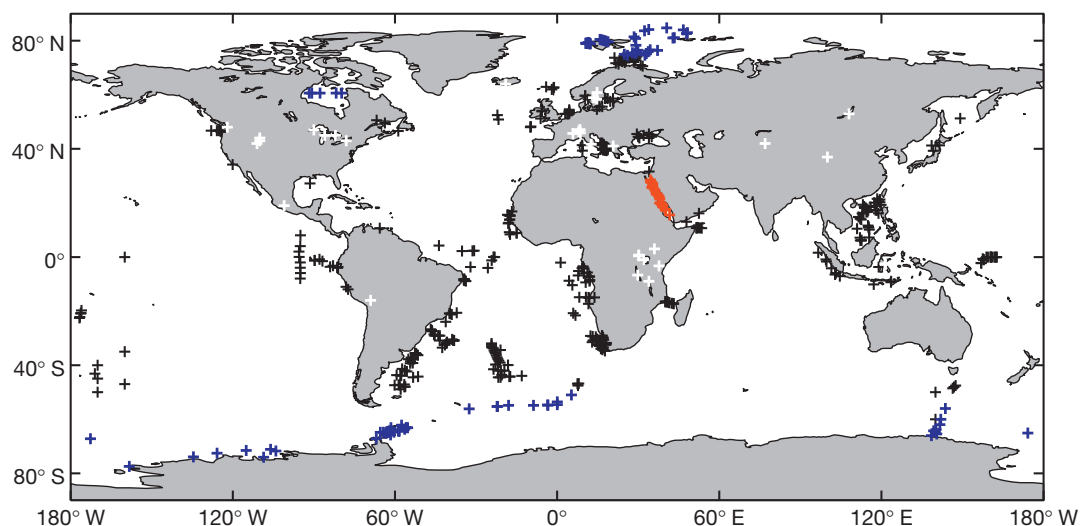
Unlike TEX<sub>86</sub>, this index does not include the crenarchaeol regioisomer. This index is referred to as ‘TEX<sub>86</sub><sup>L</sup>’ as it includes core-top data from the polar oceans and is arguably more suitable for calibration given cold ocean temperatures (<15 °C; Kim et al., 2010). Its calibration to temperature (eqn [7], Table 1) carries a higher root mean square error (RMSE) than previous calibrations but accommodates all core-top data except those from the Red Sea. Noting that TEX<sub>86</sub> data from polar regions still show a large scatter in relation to SST, Kim et al. (2010) also tested for the best index after limiting the TEX<sub>86</sub> data from 5 to 30 °C. In this case, the most robust index was simply the logarithm of TEX<sub>86</sub>. Kim et al. (2010) argued that this index, ‘TEX<sub>86</sub><sup>H</sup>’ was more appropriate for reconstructing SSTs above 15 °C. The TEX<sub>86</sub><sup>H</sup> calibration to SST is listed in Table 1 (eqn [8]).

Early investigations of TEX<sub>86</sub> in lakes found that core-top sediment values fell along the marine calibration line of Schouten et al. (2002) (Powers et al., 2004, 2005), but more recently, lake-specific calibrations were formed, recognizing that lacustrine Thaumarchaeota likely behave differently than marine Thaumarchaeota (Powers et al., 2010; Tierney et al., 2010a). Tierney et al. (2010a) found that the Powers et al. (2010) calibration (Table 1, eqn [9]) produced unrealistically high LSTs in Lake Tanganyika and thus proposed a calibration specific for tropical lakes, limiting the Powers et al. (2010) core-top dataset to sites where LST exceeded 10 °C and adding several additional tropical sites. This calibration has a shallower slope (Table 1, eqn [10]) reflecting enhanced sensitivity of TEX<sub>86</sub> at warmer temperatures, as has been observed in the marine core-top data.

Figure 3 shows the locations of all core tops measured for TEX<sub>86</sub> at the time this chapter went to press, which includes the calibration datasets of Kim et al. (2008, 2010) and additional data from the Red Sea (Trommer et al., 2009), Adriatic Sea (Leider et al., 2010), Pacific Ocean (Chazen, 2011; Ho et al., 2011; Hu et al., 2012), West Antarctica (Shevenell et al., 2011), South China Sea (Jia et al., 2012), Mozambique Channel (Fallet et al., 2012), Late Quaternary TEX<sub>86</sub> SST studies (Castañeda et al., 2010; Richey et al., 2011; Seki et al., 2009; Shintani et al., 2010), and freshwater lakes (Blaga et al., 2009; Powers et al., 2010; Tierney et al., 2010a). Although the collection and analysis of core tops is ongoing, the current data are heavily biased toward continental margins due in part to the prevalence of high sedimentation rates that allow core tops to better represent modern conditions. Open-ocean areas are not well represented by current TEX<sub>86</sub> calibrations, including the North Pacific, Indian, and North Atlantic Ocean basins. Future studies will need to target pelagic environments to improve the predictive capability of the proxy.

Figure 4 shows scatter plots between each functional form of TEX<sub>86</sub> (TEX<sub>86</sub>, 1/TEX<sub>86</sub>, TEX<sub>86</sub><sup>H</sup>, and TEX<sub>86</sub><sup>L</sup>) and SSTs to





**Figure 3** Locations of surface-sediment core tops used to calibrate TEX<sub>86</sub> to SST, including data from Kim et al. (2008, 2010), Ho et al. (2011), Trommer et al. (2009), Chazen (2011), Leider et al. (2010), Seki et al. (2009), Castañeda et al. (2010), Fallet et al. (2012), Jia et al. (2012), Hu et al. (2012), Shevenell et al. (2011), Richey et al. (2011), Shintani et al. (2010), Powers et al. (2010), and Tierney et al. (2010a). Blue crosses indicate polar ocean sites where mean annual SSTs < 2.5 °C. Black crosses indicate marine sites where SSTs > 2.5 °C. Red crosses indicate core tops from the Red Sea, and white crosses indicate core tops from freshwater lakes.

visualize the response of each ratio to temperature. Regardless of the form of TEX<sub>86</sub> (though it is somewhat improved with TEX<sub>86</sub><sup>L</sup>), there is a poor relationship between GDGT cyclization and SST in the polar oceans (blue data). At face value, this suggests that TEX<sub>86</sub> has a small, or even negligible response to SST at cold temperatures, consistent with the mesocosm incubations that show little change in TEX<sub>86</sub> between 5 and 10 °C (Wuchter et al., 2004). Alternatively, the scatter at cold SSTs could reflect the poor quality of the available instrumental SST data at high latitudes. Satellite measurements of SST in the polar oceans are typically scarce and confounded by seasonal sea-ice formation. Furthermore, much of the polar data in the Kim et al. (2010) dataset have satellite SST estimates that fall below the freezing point of seawater (as low as −3 °C) and thus are clearly inaccurate.

The behavior of TEX<sub>86</sub> in the polar oceans could also reflect a spatially variable seasonal bias in the production of GDGT lipids (however, the seasonal difference in SSTs at very high latitudes is small). Thaumarchaeota are known to express seasonal cycles in abundance and GDGT production (see Section 12.14.4), and sea-ice cover potentially limits growth during part of the year. Recent attempts to reconstruct SSTs around the West Antarctic Peninsula suggest a spring temperature bias, based on empirical comparison between local core-top TEX<sub>86</sub> values and spring SST (Shevenell et al., 2011). At this location, TEX<sub>86</sub> appears to linearly respond to changes in SST from 0.9 to 2.5 °C (Shevenell et al., 2011), demonstrating that TEX<sub>86</sub> can be applied in environments with very cold SSTs but that a seasonal and/or local calibration may be needed.

Lake core-top data generally plot within the scatter shown by the marine data, except for the warm tropical lakes that show an enhanced sensitivity to temperature (Figure 4). These include Lakes Malawi and Tanganyika, the only lakes in which TEX<sub>86</sub> has been used to reconstruct past temperatures (Powers et al., 2005; Tierney et al., 2008, 2010a). TEX<sub>86</sub><sup>L</sup> indices from these lakes are

distinctly different from marine data (Figure 4), suggesting that the crenarchaeol regioisomer plays an important role in temperature prediction in warm lakes. The different behavior of the lake TEX<sub>86</sub> data at the warm end of the calibration range justifies the need for lake-specific calibrations (Powers et al., 2010; Tierney et al., 2010a) and could also indicate that distinct archaeal assemblages with distinct temperature responses reside in freshwater environments.

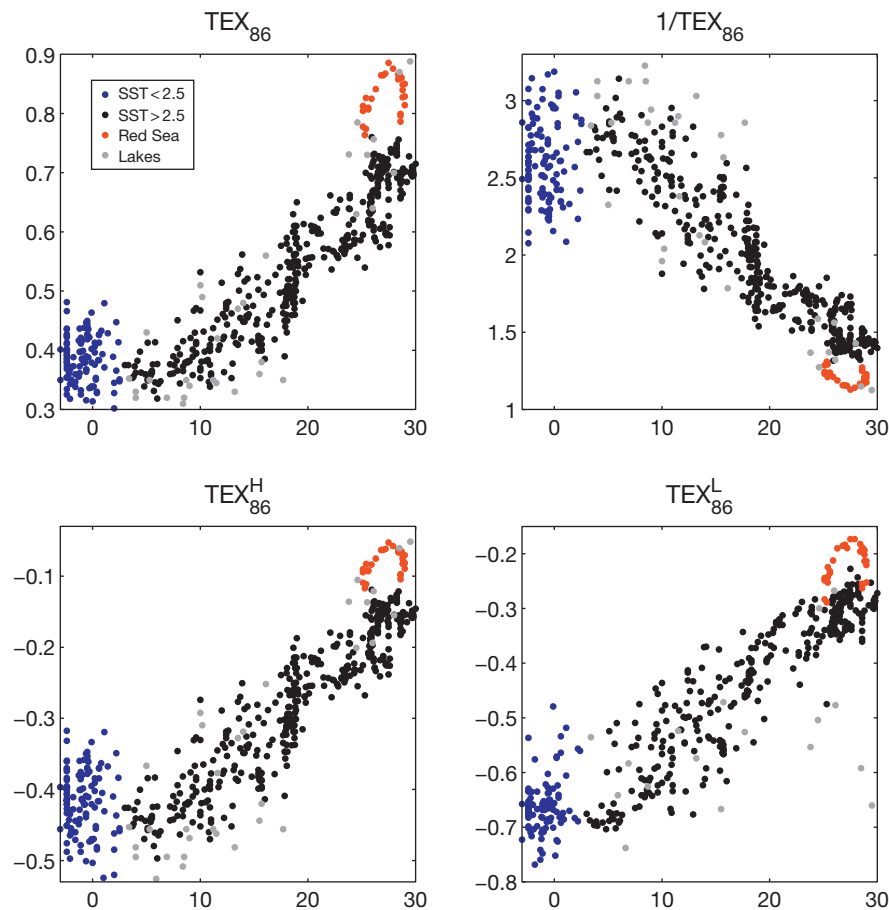
Figure 4 also highlights the unusual composition of core tops from the Red Sea (in red) where core-top GDGT distributions are significantly ( $p < 0.0001$ ) different from TEX<sub>86</sub> values from the open ocean (in black) at comparable SSTs. In addition, the horseshoe-like shape of the data strongly suggests behavior unrelated to SST, including the mixing of different populations of Thaumarchaeota (Trommer et al., 2009). There are, therefore, legitimate reasons why polar ocean and Red Sea data are distinct from other ocean settings and should be excluded from a global calibration (Kim et al., 2008, 2010). On the other hand, it could be argued that no data should be excluded, as the data collectively represent our best representation of the response of TEX<sub>86</sub> to SST given a variety of environments and analytical errors in both the TEX<sub>86</sub> estimates and SST estimates (Liu et al., 2009). The latter is particularly valid if the modern TEX<sub>86</sub> calibration is extrapolated to warmer temperatures characteristic of ancient greenhouse climates (e.g., the Cretaceous tropical oceans) – times when isolated ocean basins were characteristic and potentially harbored unique archaeal populations, like those that reside within the Red Sea today.

The proliferation of calibrations for TEX<sub>86</sub> reflects the relative infancy of the proxy and the active efforts by the organic geochemistry community to develop the best statistical model for the prediction of SST. However, for the paleoclimate researcher, it is difficult to determine which of the many calibrations to employ, and they do not in themselves provide an indication of performance. With both of the epistemological

approaches toward calibration in mind (inclusion vs. exclusion of the polar ocean and Red Sea data), I consider two cases to provide a basic evaluation of the performance of each of the proposed TEX<sub>86</sub> calibrations. The first case (Case 1) includes all available core-top data (except those from lakes, which arguably represent a different calibration problem). The second case (Case 2) excludes the Red Sea data where different archaeal communities are recognized and the polar ocean data where SSTs are lower than 2.5 °C, beyond which point there is empirically little sensitivity of TEX<sub>86</sub> to changes in SST (Figure 4). I then perform a simple twofold cross-validation to assess the predictive capability of each form of TEX<sub>86</sub>

(Figure 4), assuming a linear relationship with SST. In this procedure, one-half of the core-top data are randomly removed and the remainder are used to predict the SST of the withheld data. The RMSE is then calculated as a representation of the performance of the linear model. One thousand iterations were performed to gain an assessment of the uncertainty in the RMSE. I also perform this test after limiting SSTs within the Case 2 scenario to below 15 °C and above 15 °C, to determine whether certain functional forms perform better at low and high SSTs, respectively.

Results of the cross-validation tests are given in Table 2. When all core-top data are considered (Case 1), TEX<sub>86</sub><sup>L</sup> performs



**Figure 4** Four proposed functional forms of TEX<sub>86</sub>, including reciprocal and log transforms (TEX<sub>86</sub><sup>H</sup>) and the alternative index TEX<sub>86</sub><sup>L</sup>, plotted against SST (or LST for lake sites).

**Table 2** Results of twofold cross-validation tests, assuming a linear relationship between each proposed TEX<sub>86</sub> functional form illustrated in Figure 4

Form	Case 1	Case 2	Case 2, SSTs < 15	Case 2, SSTs > 15
TEX <sub>86</sub>	3.75 ± 0.10, N = 562	1.96 ± 0.06, N = 414	1.73 ± 0.10, N = 116	1.51 ± 0.06, N = 298
1/TEX <sub>86</sub>	3.45 ± 0.12, N = 562	1.96 ± 0.07, N = 414	1.71 ± 0.11, N = 116	1.63 ± 0.07, N = 298
TEX <sub>86</sub> <sup>H</sup>	3.49 ± 0.10, N = 562	1.90 ± 0.07, N = 414	1.72 ± 0.11, N = 116	1.54 ± 0.06, N = 298
TEX <sub>86</sub> <sup>L</sup>	2.76 ± 0.10, N = 478	2.18 ± 0.07, N = 367	1.70 ± 0.11, N = 115	1.78 ± 0.07, N = 252

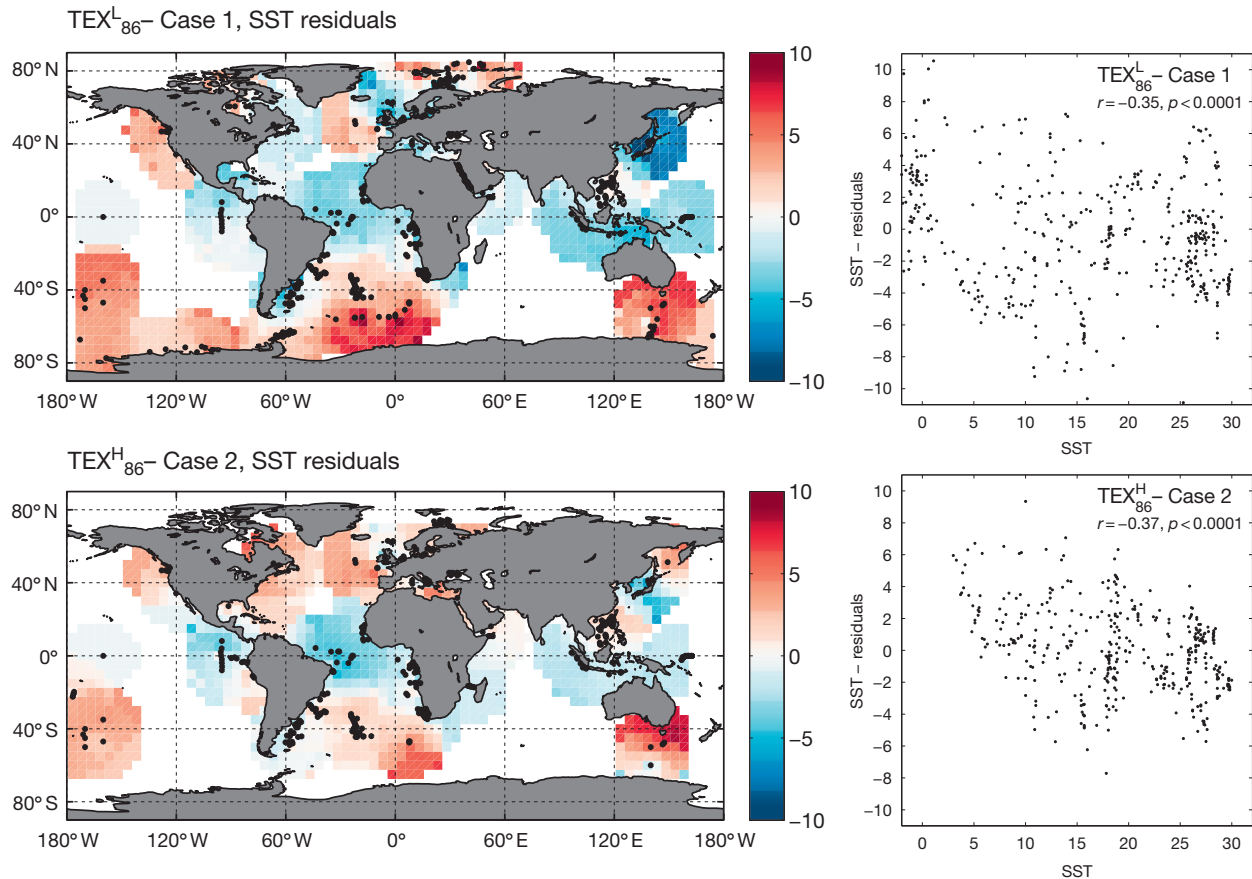
Values reported are the root mean square error (RMSE) in °C with a standard deviation based on 1000 simulations. *N* refers to the number of core-top data used. Note that assessments of TEX<sub>86</sub><sup>L</sup> use a different *N* because individual concentrations of GDGTs were not reported in all of the available core-top studies, precluding calculation of TEX<sub>86</sub><sup>L</sup>.

better than the other functional forms (although note that since absolute or fractional GDGT concentrations are not available for all core-top data, this only includes 446 rather than 512 core tops), primarily because both the polar data and Red Sea data do not deviate as severely from the remaining core-top data given the  $\text{TEX}_{86}^L$  form (Figure 4). It is also evident that forms utilizing a nonlinear transformation perform better, once again due to the nonlinear behavior introduced by the polar/Red Sea data. When the polar data and the Red Sea data are excluded (Case 2),  $\text{TEX}_{86}^L$  actually exhibits the poorest performance. If only the lower range of SSTs is considered within Case 2, all forms perform similarly. At high SSTs,  $\text{TEX}_{86}$  and  $\text{TEX}_{86}^H$  perform the best, with  $\text{TEX}_{86}^L$  resulting in slightly poorer prediction.

These results indicate that the  $\text{TEX}_{86}^L$  form is the most conservative choice as it best accommodates all core-top data. Given the restricted dataset of Case 2, both the linear and nonlinear forms of  $\text{TEX}_{86}$  produce good predictability. In formulating  $\text{TEX}_{86}^L$  and  $\text{TEX}_{86}^H$ , Kim et al. (2010) argued that  $\text{TEX}_{86}^L$  should be used at SSTs below  $15^\circ\text{C}$ , a recommendation made on the basis of including all polar ocean data. For our Case 2 scenario, where only data with SSTs above  $2.5^\circ\text{C}$  are considered, there is no compelling reason to use  $\text{TEX}_{86}^L$  when SSTs are low. Rather,  $\text{TEX}_{86}^H$  and the original linear form of

$\text{TEX}_{86}$  appear universally applicable across SSTs ranging from  $2.5$  to  $30^\circ\text{C}$ , although more high-quality calibration data from polar regions are needed to verify this conclusion.

While cross-validation is a useful metric of calibration performance, it does not highlight potential systematic biases in the calibrations that reflect the influences of oceanography and biogeochemistry. Investigating spatial patterns of calibration residual errors is one way to detect this possibility (Kim et al., 2008; Prahl et al., 2010). The spatial distribution of residual errors (Figure 5) reveals that both  $\text{TEX}_{86}^L$  and  $\text{TEX}_{86}^H$ , as they are calibrated to SST, tend to underestimate SSTs in the tropics and overestimate at the poles, particularly in the high southern latitudes. Significant negative correlations between the residual errors and SST further illustrate this tendency (Figure 5). This systematic latitudinal bias could reflect a number of factors. One possibility is that prior assumptions regarding the response of  $\text{TEX}_{86}$  to temperature are incorrect; that is,  $\text{TEX}_{86}$  is assumed to respond either exponentially or linearly in relation to SST (a linear fit of  $\text{TEX}_{86}$  to SST also exhibits a similar residual trend:  $r = -0.40$ ,  $p < 0.0001$ ). Alternatively, it could reflect an artifact of application of ordinary least squares regression, where error is assumed to occur only in the dependent variable, and such errors are assumed to be constant, independent, and normally distributed. With  $\text{TEX}_{86}$  as the

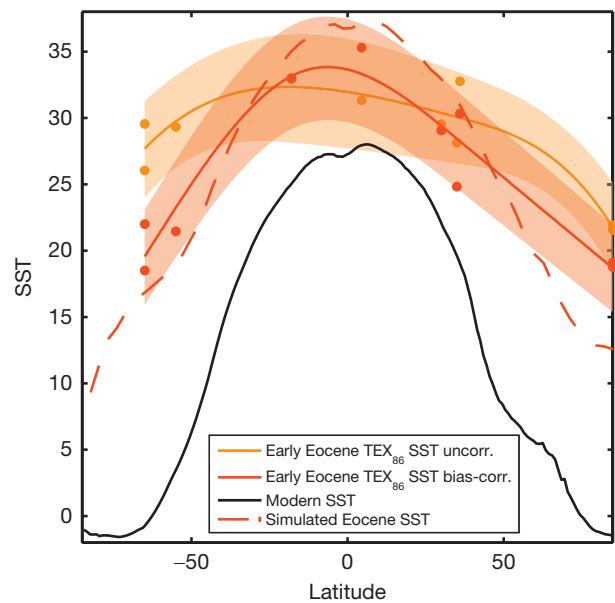


**Figure 5** Spatial maps of residual errors (predicted-observed, at left, in  $^\circ\text{C}$ ) for linear calibration of  $\text{TEX}_{86}^L$  and  $\text{TEX}_{86}^H$  to SST. To aid visualization, residual values of individual site locations (in black) were interpolated using ordinary kriging with a target grid cell size of  $5^\circ \times 5^\circ$  and a search radius of  $20^\circ$  using Gstat (Pebesma and Wesseling, 1998). Scatter plots of residual errors versus SST (at right) illustrate a persistent negative trend and significant negative correlations, reflecting a systematic underestimation of SST in the tropics and overestimation at the high latitudes.

independent variable, as both the TEX<sub>86</sub><sup>L</sup> and TEX<sub>86</sub><sup>H</sup> calibrations assume (see the preceding text), this effectively means that SST is assumed to have (normally distributed and independent) error, but not the TEX<sub>86</sub> measurements. In reality, both TEX<sub>86</sub> and SST measurements contain error, and errors in SST measurements likely vary across the SST field.

It is also possible that the latitudinal bias reflects actual biogeochemical variability, such as differences in the seasonality of production of GDGTs. For example, if GDGT production were skewed toward warmer months in the high latitudes and cooler months in the tropics, then calibration to mean annual SST as is performed here would lead to the observed residual pattern. Interestingly, in spite of the observed subsurface behavior of TEX<sub>86</sub> in upwelling zones, such as the Arabian Sea and off of West Africa (see Section 12.14.4), there does not seem to be a consistent bias of TEX<sub>86</sub> in upwelling areas in the calibration dataset, or else it is overwhelmed by the latitudinal bias (Figure 5).

Regardless of the origin of the geographic biases in the TEX<sub>86</sub> calibration, they are clearly systematic rather than randomly distributed (Figure 5) and thus likely affect temperature reconstructions – especially spatial reconstructions – and our interpretations of past temperature gradients. This is especially the case for deep-time paleoclimatic studies where, for reasons of sedimentary conditions or otherwise, TEX<sub>86</sub> may be the only proxy for SST available. For example, many of the SST estimates during the early Eocene, when atmospheric *p*CO<sub>2</sub> levels may have reached approximately 2000 ppmv, rely on the TEX<sub>86</sub> proxy (Bijl et al., 2009; Hollis et al., 2009; Keating-Bitonti et al., 2011; Liu et al., 2009; Pearson et al., 2007; Sluijs et al., 2006, 2011; Tipple et al., 2011; Zachos et al., 2006). TEX<sub>86</sub> predicts high southern latitude (55–65°S) SSTs of approximately 30 °C both during the PETM and the early Eocene (Hollis et al., 2009; Sluijs et al., 2011) and tropical temperatures of ~33 °C (Pearson et al., 2007), implying extremely small equator-to-pole temperature gradients (Figure 6) that are difficult to reconcile with basic physical assumptions about the global climate system. However, the southern high-latitude sites are located near Tasmania and New Zealand, where TEX<sub>86</sub> proxy residual error today is approximately 8 °C, among the highest observed in the global dataset (Figure 5). We cannot be certain that the geographic biases inherent in the TEX<sub>86</sub> proxy data are meaningful during the Eocene when continental configurations were different, but by the same token, we cannot be sure that modern TEX<sub>86</sub> calibrations are appropriate for the Eocene either. If we correct the early Eocene data for the modern geographic biases based on the spatial residual error distribution in Figure 5, we find that the tropical estimates are slightly warmer and the high southern latitude estimates in particular are much cooler, closer to 20 °C (Figure 6). The resulting modeled latitudinal gradient in temperature is still more equable than today's climate (Figure 6) but perhaps does not require a fundamental reassessment of heat transport. Climate model simulations of the early Eocene driven with high *p*CO<sub>2</sub> concentrations can produce polar temperatures of 10–15 °C and tropical temperatures near 35 °C (Huber and Caballero, 2011) that are in rough agreement with the corrected TEX<sub>86</sub> data (Figure 6). Such simulations require *p*CO<sub>2</sub> of approximately 4500 ppmv, which is on the higher end of the span of proxy estimates



**Figure 6** Early Eocene (55–48 Ma, including Paleocene–Eocene thermal maximum data) latitudinal SST gradients based on uncorrected TEX<sub>86</sub> data versus geographically biased corrected TEX<sub>86</sub> data. The TEX<sub>86</sub> data were corrected using regional median residual values based on the data shown in Figure 5. Data are from Sluijs et al. (2006), Zachos et al. (2006), Pearson et al. (2007), Hollis et al. (2009), Liu et al. (2009), Bijl et al. (2009), Sluijs et al. (2011), Keating-Bitonti et al. (2011), and Tipple et al. (2011). All data were calibrated to SST using the TEX<sub>86</sub><sup>L</sup> form and its linear regression to SST based on the Case 2 scenario outlined earlier. Latitudinal gradients were modeled using a stiff cubic spline. Error bars represent the 1 $\sigma$  uncertainty based on the standard deviation of the proxy data about the mean compounded with calibration uncertainty. The modern SST gradient is based on World Ocean Atlas 2009 data (Locarnini et al., 2010). The Eocene SST gradient is from a climate model simulation performed with the NCAR CCSM3 OAGCM given Eocene topography and *p*CO<sub>2</sub> concentrations of 4480 ppmv (Huber and Caballero, 2011).

(Zachos et al., 2008), potentially indicating that Eocene Earth system sensitivity is higher than assumed in most climate models.

## 12.14.7 Conclusion

This chapter not only provides an overview of the TEX<sub>86</sub> proxy but also attempts to explore its limitations, as the study of GDGTs in the marine realm is ongoing and there is much left to learn. TEX<sub>86</sub>'s unique contribution to paleoclimate is that it allows for unprecedented reconstructions of global and regional temperatures in both space and time, and therefore can be used to make important inferences concerning past temperature variability and Earth system sensitivity (e.g., Jenkyns et al., 2012; Littler et al., 2011; Liu et al., 2009; Royer et al., 2012; Schouten et al., 2003; Sluijs et al., 2006; Tierney et al., 2010a; Zachos et al., 2006). However, uncertainties in the proxy are at present difficult to fully constrain. First and foremost, establishing confidence in TEX<sub>86</sub>-temperature reconstructions will require a more complete understanding of



archaeal ecology in the global ocean. Unlike the alkenones that comprise the marine U<sub>37</sub><sup>K'</sup> proxy, GDGTs are produced by a large diversity of archaeal species and the empirical relationship between TEX<sub>86</sub> and temperature seems to reflect a community, rather than single-species, adaptation. Second, better constraints on the export and preservation of GDGTs in sediments are needed to resolve outstanding issues surrounding the sedimentary TEX<sub>86</sub> signal, including whether certain seasons or water depths are preferentially recorded. Finally, a re-examination of both the theoretical and statistical assumptions behind the existing calibrations will be critical to improve regression models and the ability of such models to accurately predict ancient SSTs.

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