Distributional variations in marine crenarchaeotal membrane lipids:
a new tool for reconstructing ancient sea water temperatures?

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Abstract

It has recently been shown that membrane lipids of marine crenarchaeota, a ubiquitous and abundant component of plankton, occur in relatively high concentrations in recent and ancient sediments. In this study we investigated the environmental controls on the relative distribution of these lipids in surface sediments. We especially focussed on temperature, as it is known from the thermophilic genetic relatives of marine crenarchaetoa that the composition of their membrane strongly depends on growth temperature. Indeed, a significant linear correlation ($r^2 = 0.92$) is found between the number of cyclopentane rings in sedimentary membrane lipids derived from marine crenarchaeota and the annual mean sea surface temperatures. This suggests that the mechanism of physical adaptation of their membrane compositions to temperature is identical to that of their thermophilic relatives. In turn, archaeal lipid distributions in sediments may thus allow the reconstruction of sea water temperatures of ancient marine environments.
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1. Introduction

The determination of past sea surface temperatures (SST) is of primary importance for reconstructing climatic changes, such as the last glacial maximum, or the impact of regularly occurring phenomena on local climate. Several geochemical proxies for SST reconstructions have been developed over the last decades and the most commonly used include $\delta^{18}O$ and Mg/Ca ratios of planktonic foraminifera [1] and the $U^K_{37}{}'$ ratio of long-chain unsaturated ketones from haptophyte algae (e.g. [2,3]). However, several problems exist with the current SST proxies. For instance, reconstructions of SST based on the analysis of planktonic foraminifera are complicated by uncertainties regarding depth habitat of species, state of

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preservation of the shell, carbonate concentrations and the original oxygen isotope composition of sea water (e.g. [4]). SST reconstructions using alkenones are somewhat more robust in this respect although questions remain on their diagenetic stability [5] and it is still unclear what their biochemical role in their source organism, haptophyte algae, is. In addition, in some settings they do not occur in substantial amounts and they are generally not applicable beyond ca. 6 Ma ago [3]. Hence, there is still a need for additional SST proxies, which can be used in combination with more established SST proxies to more accurately reconstruct paleotemperatures [3].

Besides alkenones, the distribution of a number of other lipids present in organisms, especially in their membranes, are known to vary with growth temperatures. One of the best examples are specific membrane lipids biosynthesized by (hyper)thermophilic archaea, glycerol dialkyl glycerol tetraethers (GDGTs), which can contain up to eight cyclopentane rings [6]. Culture experiments have shown that the relative distribution of cyclopentane rings in the GDGTs strongly depends on culture temperatures [6–8]. For example, the weighted average number of cyclopentane rings in GDGTs of *Thermoplasma acidophilum* increased from 1.6 at 40°C to 2.1 at 60°C [8]. This physical adaptation is due to the higher thermal transition points of cell membranes composed of GDGTs with cyclopentane rings [7]. Hence, the biosynthesis of cyclopentane rings in the membrane lipids of thermophilic archaea is generally considered to be a mechanism for temperature adaptation of the membrane.

Interestingly, the kingdom crenarchaeota of the domain archaea were formerly thought to consist only of hyperthermophilic organisms living at temperatures > 60°C. However, phylogenetically related crenarchaeota are also present in oceans and lakes with environmental temperatures ranging from 0 to 30°C [9–13]. In fact, one specific clade within the crenarchaeota is even thought to make up 20% of the picoplankton in the world's oceans [9]. A recent study showed that the abundant 'cold' crenarchaeota from the marine water column biosynthesize similar GDGTs as those encountered in cultured hyperthermo-

philic archaea with the exception of GDGT **I** ('crenarchaeol'; Fig. 1), which is uniquely characterized by the presence of a cyclohexane ring [14,15]. Crenarchaeol is, thus, a specific marker for 'cold' crenarchaeota. The biosynthesis of crenarchaeol by marine crenarchaeota is likely an adaptation to their 'cold' environments in comparison with their (hyper)thermophilic relatives. Molecular modeling has shown that crenarchaeol prevents dense packing of the membrane of the 'cold' crenarchaeota at normal temperatures (i.e. 25°C) and thus enables these organisms to live at 'low temperatures' in comparison with their thermophilic relatives [15].

The above results suggest that temperature may be the main controlling factor in determining the membrane composition of marine crenarchaeota and thus that their lipid distribution may reflect environmental temperatures. Here we report the analysis of GDGTs derived from marine crenarchaeota in globally distributed core top sediments and show that their distribution is highly correlated with SST. This suggests that GDGT analysis of recent and ancient sediments may be a novel tool to reconstruct sea water temperatures.

2. Methodology

Typically, freeze-dried sediments were ultrasonically extracted (ca. 1 min) three times with methanol, three times with dichloromethane (DCM)/methanol (1:1, v/v), and three times with DCM and all extracts were combined. The bulk of the solvent was removed by rotary evaporation under vacuum. For GDGT analyses, extracts were further purified by column chromatography using Al_2O_3 as stationary phase and a DCM/methanol (1:1, v/v) mixture as the eluent. The obtained fraction was then dissolved by sonication (5 min) in hexane/propanol (99:1, v/v). The resulting suspension was centrifuged (1 min, 3500 rpm) and the supernatant filtered through a 0.45 μm, 4 mm diameter PTFE filter prior to analysis by high performance liquid chromatography/atmospheric pressure positive ion chemical ionization mass spectrometry (HPLC/APCI-MS). Con-
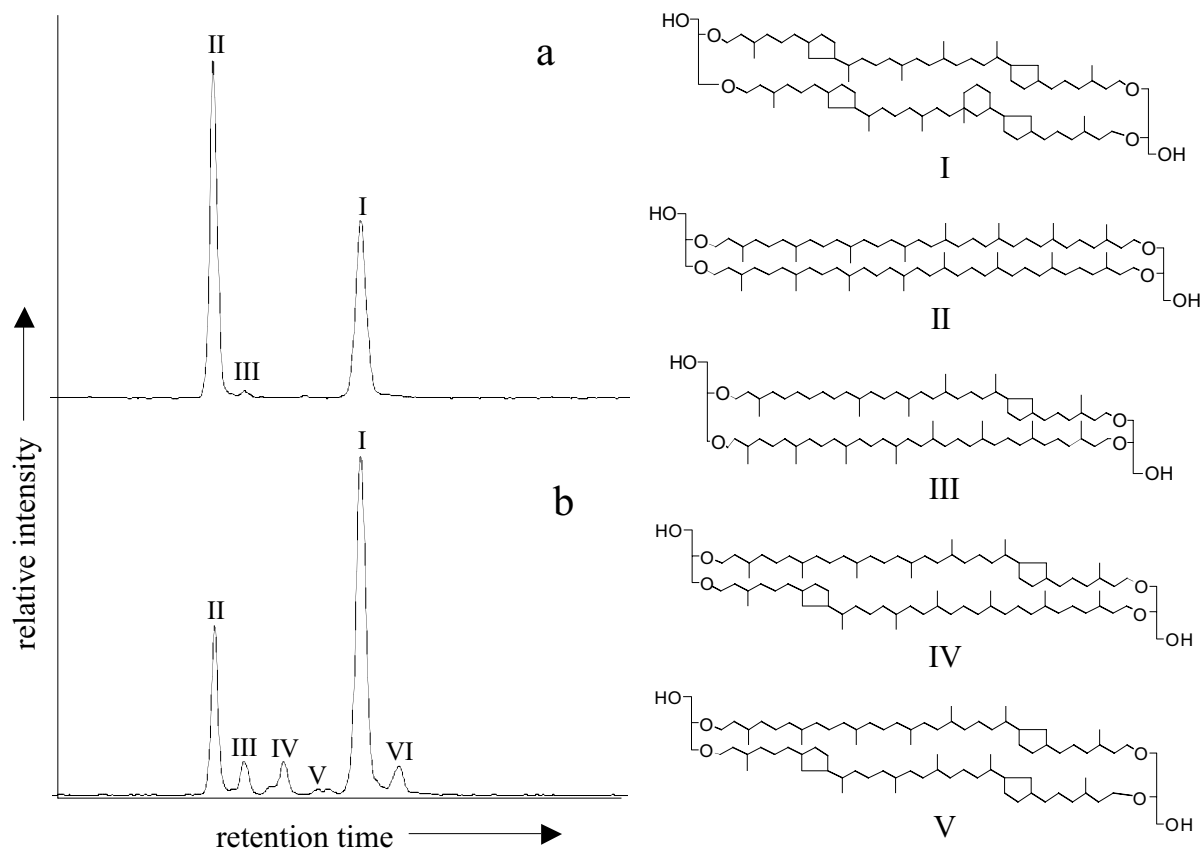
Fig. 1. HPLC/MS base peak chromatograms of (a) a surface sediment from Halley Bay Station (Antarctica), (b) a surface sediment from the Arabian Sea. Roman numerals indicate structures drawn besides. GDGT-0 **II** and GDGTs **III**–**V** were identified based on standards obtained from a lipid extract of *Sulfolobus solfaticarus* with known composition [14]. Crenarchaeol **I** was identified by isolation and analysis by high-field ^{13}C-NMR [15]. GDGT **VI** has an, as yet, unknown structure but contains five rings, as deduced from its positive ion APCI mass spectrum. Furthermore, HI cleavage of an isolated fraction of a sediment extract containing high amounts of **VI** released carbon skeletons with similar mass spectral features as those of carbon skeletons released from **I**. Thus, it is thought that **VI** is an isomer of **I**.

ditions for HPLC-MS analyses of the purified extracts were as described earlier [16]. Quantitation was achieved by integration of the peak areas in the respective $[M+H]^+$ and $[M+H]^++1$ (i.e. protonated molecule and isotope peak) ion traces of GDGTs. For C_{37} alkenone analyses, extracts were derivatized with diazomethane, eluted over a silica gel column using ethyl acetate as the eluent, and silylated with BSTFA/pyridine. The derivatized fraction was analyzed by gas chromatography flame ionization detection (GC-FID) and mass spectrometry. The $U^K_{37}{}'$ was determined by integrating the peak areas of the $C_{37:2}$ and $C_{37:3}$ alkenone in the GC-FID trace. Duplicate analysis of a

number of sediment samples containing abundant alkenones revealed that the accuracy of the $U^K_{37}{}'$ values reported here is ca. ± 0.01.

3. Results and discussion

3.1. Environmental controls on GDGT distribution

To determine the controlling environmental factors on GDGT distributions we analyzed over 40 surface sediments (mostly 0–2 cm) from 15 different worldwide locations (Fig. 2). We initially use surface sediments rather than water col-
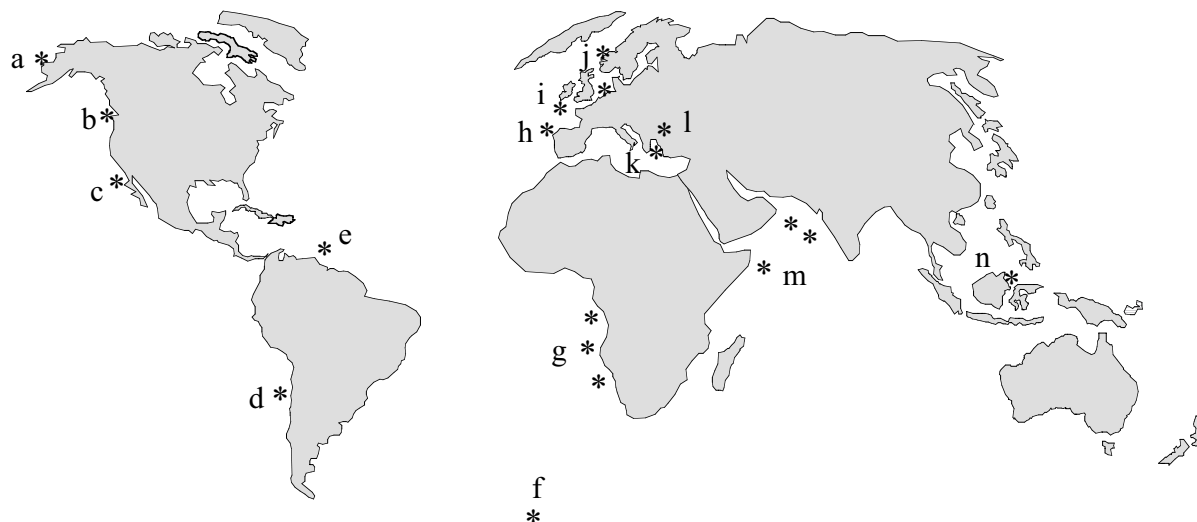
Fig. 2. Surface sediments analyzed in this study. Samples are from (a) Skan Bay (*1*), (b) Saanich Inlet (*1*), (c) Santa Monica Basin (*1*), (d) Peru Margin (*2*), (e) Cariaco Basin (*1*), (f) Halley Bay Station (*1*), (g) Angola Basin (*24*), (h) Iberian Margin (*2*), (i) Wadden Sea (*3*), (j) Skagerrak and Drammensfjord (*2*), (k) Aegean Sea (*1*), (l) Black Sea (*1*), (m) Arabian Sea (*3*) and (n) Kau Bay (*1*). Numbers in italics between brackets indicate the number of samples analyzed from this area.

umn filtrates since the latter only provide a 'snapshot' view on GDGT distribution, whilst surface sediments provide a time-integrated picture [17,18]. The sample locations range from shallow coastal waters like the Wadden Sea (10–30 m water depth) to open ocean settings like the Arabian Sea (>1500 m water depth) and the Black Sea (>2000 m water depth). Furthermore, there are large gradients not only in annual temperatures (2–27°C) but also in annual salinities (20–35‰) and oxicity, i.e. completely oxygenated waters in the Wadden Sea or large anoxic water columns such as in the Black Sea. The data set also comprises upwelling areas (e.g. eastern Atlantic Ocean, Arabian Sea, Peru Margin) and a number of non-upwelling areas (e.g. Black Sea, Wadden Sea, Saanich Inlet). Hence, the data set represents a large set of diverse oceanic provinces.

A striking observation is that GDGTs in surface sediments from cold areas (e.g. Antarctic waters; Fig. 1a) consisted almost completely of GDGT **II** and crenarchaeol (**I**), whilst the GDGT distributions in surface sediment from warmer areas (e.g. Arabian Sea; Fig. 1b) are dominated by crenarchaeol and contained rela-

tively higher amounts of additional structural isomers. These latter isomers are the one to three cyclopentane ring-containing GDGTs (**III–V**) and an isomer of crenarchaeol (**VI**). This is in complete accordance with what is observed in the (hyper)thermophilic genetic relatives of marine crenarchaeota, i.e. the weighted average number of cyclopentane rings in GDGTs substantially increases with growth temperature [6–8]. It thus seems that the marine 'cold' crenarchaeota have the exact same physiological mechanism to adapt their membranes to temperature, i.e. via the biosynthesis of cyclopentane rings in the membrane lipids. This ability may also explain why they can thrive at such a large range of temperatures, i.e. between 0 and 30°C.

The fact that the distribution of GDGTs changes with sea water temperatures does not provide *direct* evidence that temperature is the controlling factor. For example, salinities also vary to a significant degree and are positively correlated to temperature. However, to the best of our knowledge there are no reports that relative GDGT distributions in archaea are depending on salinity. In fact, halophilic archaea living at high salt concentrations do not seem to contain

GDGTs but rather glycerol diphytanyl diethers [19]. Other environmental factors seem much less likely to affect the *relative* distribution of GDGTs although they may substantially affect the absolute concentrations of GDGTs. Nutrient concentrations will certainly influence the relative abundance of crenarchaeota and thus affect GDGT concentrations. Significant negative correlations were found between concentrations of nitrate and abundance of crenarchaeotal cells [20]. This may thus determine the absolute abundance of GDGTs but is less likely to affect the relative abundance of GDGTs. Thus, based on the extensive literature on GDGT behavior in genetically related archaea and the well-studied physical properties of the lipids, it is most likely that the distribution of GDGTs is controlled by growth temperature.

3.2. Quantification of distributional changes

To quantitatively express the control of temperature on GDGT distribution, we compared several different ratios of GDGTs with the local annual mean SST (from [21]). We use the annual mean SST at this point as it is still unknown where and when the marine crenarchaeota are thriving in the water columns of the environments investigated (see discussion below). Similar to the way microbiologists quantitatively express the change in lipid distribution in (hyper)thermophilic archaea with growth temperature [6–8], we first calculated the weighted average number of cyclopentane rings found in GDGTs **I**–**VI** in surface sediments. It varied from 1.4 to 3.3 and there was a linear correlation with SST ($r^2 = 0.64$) (Fig. 3a). Other linear correlations were found between the ratio of GDGT **II** versus crenarchaeol plus GDGT **II** ($r^2 = 0.62$) and the ratio of GDGTs **III**–**VI** versus total amounts of GDGTs ($r^2 = 0.68$). However, the best fit was obtained by the TEX$_{86}$ index (TetraEther indeX of tetraethers consisting of 86 carbon atoms) defined as:

$$\text{TEX}_{86} = \frac{([\mathbf{IV}] + [\mathbf{V}] + [\mathbf{VI}])}{([\mathbf{III}] + [\mathbf{IV}] + [\mathbf{V}] + [\mathbf{VI}])} \tag{1}$$
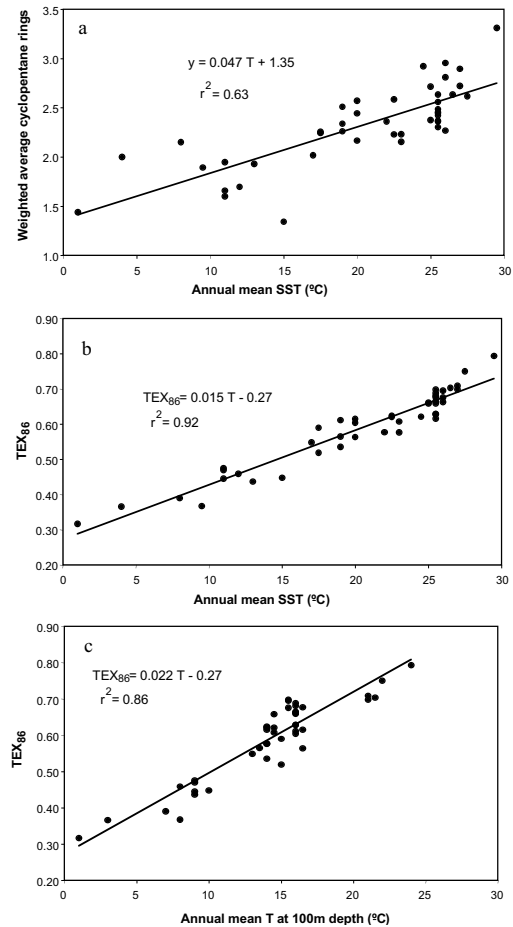
Correlation of this index to the annual mean

Fig. 3. Correlation of the geochemical proxies with SST. Graph a shows the correlation of the average number of cyclopentane rings in GDGTs **I**–**VI** with annual mean SST determined from [21] with a precision of 0.5°C. Graph b shows the correlation of TEX$_{86}$ (defined as in Eq. 1) from surface sediments with annual mean SST. Graph c shows the correlation of TEX$_{86}$ from surface sediments with annual mean temperatures at 100 m water depth (or bottom water temperatures at lower water depths of surface sediments).

SST (Fig. 3b) gave the following linear equation:

$$\text{TEX}_{86} = 0.015T + 0.28 \ (r^2 = 0.92) \tag{2}$$

with T = annual mean SST (in°C; [21]). The reason why this particular index yields the highest correlation coefficient may be two-fold. Firstly, GDGT **II** occurs not only in planktonic non-thermophilic crenarchaeota [14], but also in methanogenic archaea. However, the latter archaea do not

produce any GDGTs with cyclopentane rings [22]. Methanogens can either be present in anoxic sediments or in anoxic microniches in guts of zooplankton and sinking particles [23] and may, thus, contribute to the sedimentary GDGT pool. This additional contribution of GDGT **II** from methanogens may distort the temperature signal in the distribution of the GDGTs. In contrast, in the investigated settings GDGTs **I** and **III**–**VI** only derive from marine crenarchaeota. Secondly, inclusion of crenarchaeol **I** into the index yields poorer correlations with SST since its concentration is an order of magnitude higher than GDGTs **III**–**VI**, thereby substantially diluting their impact on the index. Whilst crenarcheaol is biosynthesized to adapt the membrane of marine crenarchaeota to growth temperatures lower than that of (hyper)thermophilic archaea (i.e. < 40°C), GDGTs **III**–**VI** may be used as a means to optimally thrive within the range of 0–30°C.

Thus, based on Eq. 2 it is now possible to quantitatively reconstruct sea water temperatures by the analysis of tetraether lipid distributions. It should be mentioned that TEX_{86} values are more difficult to determine in low-temperature regions (< 10°C), where the relative abundances of GDGTs **III**–**VI** are much lower compared to GDGTs **I**–**II**. However, in regions with higher temperatures the relative abundances of GDGTs **III**–**VI** are sufficiently high to allow accurate determinations of their concentrations and hence the TEX_{86} value. In comparison, $U_{37}^{K'}$ values are less accurate in regions where it approaches 0 or 1 due to the low concentrations of the $C_{37:2}$ and $C_{37:3}$ alkenones, respectively (e.g. [3,17]). One further restriction of the application of TEX_{86} is that it cannot be used in sites with high rates of anaerobic oxidation of methane. High amounts of ^{13}C-depleted GDGTs **II**–**IV** have been found in these sediments and they are derived from anaerobic methanotrophic archaea (e.g. [24,25]). However, this phenomenon is highly localized and is unnoticeable less than a few meters from the sites with high oxidation rates [24,25]. Furthermore, in water columns where anaerobic oxidation of methane takes place no disturbance of the marine crenarchaeotal TEX_{86} signal is noticed (see below).

3.3. Accuracy of temperature reconstructions

An important point for temperature proxies is the relative accuracy with which temperatures may be reconstructed. Using duplicate analysis of 17 sediment samples the current accuracy of the LC/MS method for TEX_{86} analysis was estimated to be ± 0.022. The standard error in the temperature estimate using the current TEX_{86} calibration (Eq. 2) is ± 2.0°C. Clearly, further improvements in methodology are required to improve the accuracy of determining the TEX_{86} values and are currently underway in our laboratory. However, the method is fairly stable and not so much depending on exact LC/MS conditions as the data presented here were accumulated over a 1 year time period where columns, column flows and spraying conditions have been varied to obtain optimal separation and maximal ion signal for tetraether lipids. Hence, it seems at this point unlikely that LC/MS conditions affect the slope and intercept of the correlation line potentially making it a generally applicable method in many laboratories.

3.4. Origin of temperature signal

The high correlation of TEX_{86} with annual climatic mean SST may suggest that the GDGT-producing crenarchaeota are predominantly living in the surface waters of the ocean. This contradicts counts of crenarchaeotal cells, using fluorescence in situ hybridization, in open ocean sites, which show that the upper 500 m of the ocean are characterized by constantly high numbers and that they only drop below this level [9,26,27]. In fact, the correlation of TEX_{86} with temperatures at water depths of 100 m (or bottom water temperatures in case of settings with a small water depth) is also high ($r^2 = 0.86$) (Fig. 3c). Hence, it remains unclear which temperature is actually reflected in the TEX_{86} proxy. One clue may be obtained by analyzing the distributions of GDGTs in suspended organic matter throughout the water column. This would enable one to detect where in the water column the temperature signal of sedimentary GDGTs is generated. Analyses of water samples in the Arabian Sea at depths varying be-

tween 35 and 1500 m show that concentrations of GDGTs **I**–**VI** are on average similar for samples from 35–500 m depths but lower at 1000–1500 m depths [27]. In the Black Sea, concentrations of crenarchaeol were the highest at ca. 130 m water depth around the chemocline and then rapidly decreased with depth [24]. Interestingly, the TEX_{86} values in suspended organic matter in both these basins were relatively constant over depth. In the Arabian Sea TEX_{86} averages 0.72 ± 0.06, which is identical to the TEX_{86} found in the surface sediment (0.71), despite temperature variations ranging from 27°C at the surface to ca. 5°C at 1500 m water depth. Similarly, in the Black Sea, TEX_{86} values in the upper 400 m average 0.42 ± 0.05, which is similar to that of the surface sediment (0.44) [24]. This indicates that the composition of marine crenarchaeotal membrane lipids is mainly determined by upper water column temperatures rather than the relatively invariant bottom water temperatures. It also hints at the possibility that the TEX_{86} values are not solely determined by physiological adaptation to the temperature of the surrounding water masses of one species of marine crenarchaeota. Possibly it is also influenced by genetically different populations who have adapted their membrane composition to the specific environments in which they live. We are currently investigating this hypothesis using water column material and mesocosms.

Initial results thus suggest that TEX_{86} does not vary to a large extent in the water column and that GDGTs are biosynthesized in the upper parts of the water column, although not necessarily only in the photic zone. What eventually determines the signal in the surface sediments is the time and origin of fluxes of archaeal lipids to the sediments. For example, Wakeham et al. [24] found high amounts of ^{13}C-depleted GDGTs in the deep anoxic zone of the Black Sea (> 1300 m water depth) but were not able to find these lipids in sediment traps or underlying surface sediments. This led them to suggest that the dominant flux of GDGTs to the surface sediments was coming from the upper part of the water column perhaps due to a lack of transport mechanism (e.g. by faecal pellets) by which lipids from deeper waters reach surface sediments. Similarly, even though

GDGTs **I**–**VI** are biosynthesized throughout the water column, the dominant signal in the surface sediments may be coming from the upper parts of the water column, where they are highest in absolute abundance and where there are efficient transport mechanisms of GDGTs to the sediment. Further research using detailed analysis of sediment traps is currently underway to shed more light on this.

3.5. Significance of seasonal factors

Further insight into the controlling factors of GDGT distribution was gained by the analysis of surface sediments from the eastern South Atlantic (between latitudes 2 and 24°S and longitudes 2 and 14°E) with annual SSTs varying between 19 and 26°C [21]. This area includes upwelling cells induced by trade winds and by river input. Besides TEX_{86} we also determined $U_{37}^{K}{}'$, the temperature-sensitive index based on the distribution of long-chain alkenones biosynthesized by haptophyte algae [2,17,28]. We did not extend this comparison to our global data set as in quite a number of sediments no C_{37} alkenones were detected. Furthermore, we wanted to investigate the effect of seasons and water depths on the different proxies and thus had to limit ourselves to a regional comparison.

Müller et al. [17] have shown that $U_{37}^{K}{}'$ correlates best with annual SST and thus it would be expected that TEX_{86} correlates with $U_{37}^{K}{}'$. Indeed there is a linear correlation but with a relatively low correlation coefficient ($r^2 = 0.50$) (Fig. 4a). Comparison of TEX_{86} and $U_{37}^{K}{}'$ with the annual mean SST of the Angola Basin area [21] showed that both correlate with these temperatures although TEX_{86} has a lower correlation coefficient than $U_{37}^{K}{}'$ ($r^2 = 0.70$ and 0.85, respectively). Possibly, this difference between the TEX_{86} proxy and $U_{37}^{K}{}'$ may be caused by the greater depth intervals at which the GDGTs are produced compared to alkenones. However, comparison of TEX_{86} with water temperatures at 100 m resulted in a linear correlation with a much reduced correlation coefficient ($r^2 = 0.36$). Interestingly, when both indices are compared to seasonal mean SST a different picture emerges. $U_{37}^{K}{}'$ correlates best
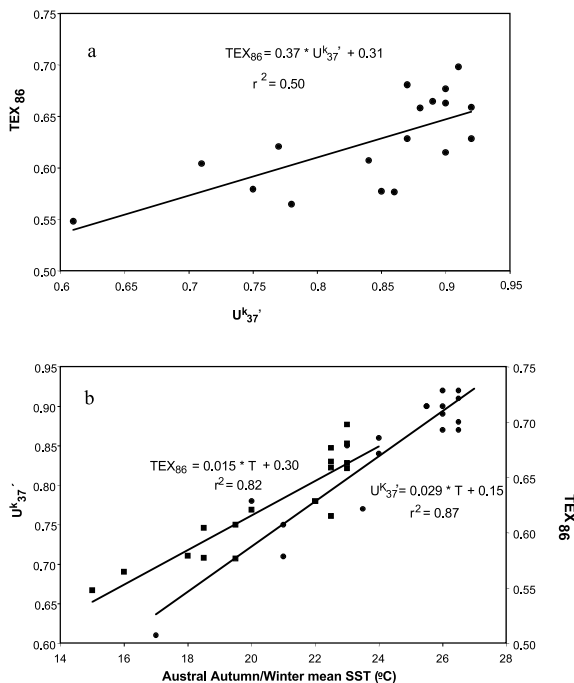
Fig. 4. Correlation of the geochemical proxies in core-top sediments with SST in the Angola Basin. Graph a shows the correlation of TEX_{86} (defined as in Eq. 1) with $U_{37}^{K'}$. Graph b shows correlations of $U_{37}^{K'}$ with austral autumn mean SST and TEX_{86} with austral winter mean SST from Angola Basin core-top sediments with SSTs determined from [21] with a precision of 0.5°C.

with austral autumn SST ($r^2 = 0.87$) (Fig. 4b). This is different from the findings of Müller et al. [17], who found no statistically different correlations between the $U_{37}^{K'}$ of eastern South Atlantic core tops and SST for the different seasons. This apparent difference may be explained by our smaller set of samples from a small part of the area investigated by Müller et al. [17] and thus our differences may not be statistically significant. Alternatively, in the region around the Angola Basin seasonal influences such as upwelling are more noticeable than for the whole eastern Southern Atlantic. In contrast to $U_{37}^{K'}$, TEX_{86} correlates well with austral winter SST ($r^2 = 0.82$) (Fig. 4b), yielding a nearly identical correlation equation as the global correlation Eq. 2. This may explain the relatively poor correlation between $U_{37}^{K'}$ and TEX_{86}; the former possibly reflects the austral autumn SST, which are similar in pattern to an-

nual SST [21], whilst the latter index may reflect more the austral winter SST. Clearly, further studies using, for example, sediment traps are needed to determine which part of the season is exactly reflected in the TEX_{86} proxy. However, these preliminary results suggest that it is unlikely that both proxies reflect the exact same sea water temperatures. Support for this hypothesis comes from molecular biological studies. In surface waters of both Antarctic coastal waters [29] and the Santa Barbara Channel [20] a significant negative correlation is found between the abundance of chlorophyll and particulate organic carbon and the abundance of crenarchaeota. This suggests that the crenarchaeota are especially abundant during times when the majority of phytoplankton is not blooming and thus it may be expected that the GDGTs biosynthesized and ending up in the sediments mostly reflect these periods. This may explain the contrasting behavior of the two SST proxies.

4. Conclusions

The establishment of a new organic geochemical temperature proxy, TEX_{86}, allows a new way of deciphering past oceanic water temperatures. GDGTs occur ubiquitously [14], including sites where alkenones are not produced due to the absence or low abundance of alkenone-producing haptophyte algae. For example, organic-rich varved sediments deposited in marine fjords, like Saanich Inlet (Canada) and the Drammensfjord (Norway), are ideal for climatic reconstructions, but SST reconstructions are difficult due to the absence of long-chain alkenones. In addition, GDGTs have been found in sedimentary rocks of at least up to 112 Myr old [30], whereas $U_{37}^{K'}$ has only been applied in sediments up to ca. 6 Myr old [31]. This novel proxy thus potentially allows the reconstruction of oceanic sea water temperatures over time periods up to the middle Cretaceous. It is clear that further research is needed before this proxy can be confidently used in paleotemperature reconstructions and research is well underway in our laboratory to investigate factors potentially influencing TEX_{86}. Neverthe-

less, the results presented here show that this new proxy has the potential to reveal new details on sea water temperatures from all kinds of ancient depositional environments.

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