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2-Methylhopanoids: Biomarkers for Cyanobacteria and for Oxygenic Photosynthesis

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50% of this area in March) is reduced. However, the increases in stratospheric halogen loading due to anthropogenic emissions has contributed significantly to the springtime decrease since the 1970s. The observed decadal decrease in column O₃ values in March north of 63° N may contain a large (>50%) contribution from slow, year-round, halogen-catalysed depletion.

These results have implications for future levels of ozone in the Arctic region. Although the amount of PSC-induced chemical depletion within the vortex may be enhanced due to stratospheric cooling²⁴, the main factor determining the average ozone column to date in a given winter is dynamics. This transport is affected by interannual variability, but may also be subject to a trend—for example, due to stratospheric O₃ decrease or other climate-related effects. Possible future circulation changes (for example, those that make the Arctic vortex more Antarctic-like) could significantly change mean wintertime ozone levels by transport alone. Also, while Shindell *et al.*²⁴ pointed out the potential feedback between stratospheric cooling which may delay the recovery of Arctic ozone as halogen levels decrease, the contribution of halogens to the decadal decrease in ozone in the larger extra-vortex region may be expected to follow the halogen loading more closely, unless cooling extends PSC activation outside the vortex. □

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2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis

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Oxygenic photosynthesis is widely accepted as the most important bioenergetic process happening in Earth's surface environment¹. It is thought to have evolved within the cyanobacterial lineage, but it has been difficult to determine when it began. Evidence based on the occurrence and appearance of stromatolites² and microfossils³ indicates that phototrophy occurred as long ago as 3,465 Myr although no definite physiological inferences can be made from these objects. Carbon isotopes and other geological phenomena^{4,5} provide clues but are also equivocal. Biomarkers are potentially useful because the three domains of extant life—Bacteria, Archaea and Eukarya—have signature membrane lipids with recalcitrant carbon skeletons. These lipids turn into hydrocarbons in sediments and can be found wherever the record is sufficiently well preserved. Here we show that 2-methylbacteriohopanepolyols occur in a high proportion of cultured cyanobacteria and cyanobacterial mats. Their 2-methylhopane hydrocarbon derivatives are abundant in organic-rich sediments as old as 2,500 Myr. These biomarkers may help constrain the age of the oldest cyanobacteria and the advent of oxygenic photosynthesis. They could also be used to quantify the ecological importance of cyanobacteria through geological time.

In the Bacteria, bacteriohopanepolyols (BHP) are amphiphilic membrane biochemicals⁶ that serve a regulating and rigidifying function similar to that of sterols in the Eukarya. The hydrocarbon skeletons of BHP are extremely refractory and resist biodegradation to become incorporated into kerogen or bound into sulphur-linked macromolecules. They also survive high geothermal gradients accompanying hydrocarbon generation and ultimately appear in petroleum as defunctionalized and stereochemically modified⁷ 'geohopanes'. Although many aspects of the preservation of BHP are well understood, there remains uncertainty about the sources of BHP and environmental controls on their abundance and distribution. This study assesses the role of cyanobacteria as a source of fossil hopanoids.

Table 1 summarizes the hopanoid contents of cultured cyanobacteria and mat communities that we analysed, along with data extracted from the literature. The high polarity and complexity of the hydrophilic side chains of BHP⁸ precludes a generalized gas chromatography–mass spectrometry (GC–MS) analysis that detects all hopanoids. Accordingly, we applied the methodology of ref. 9 (Fig. 1) in our survey of the occurrence of hopane skeletons in cultures and environmental samples. Structural variation in the hydrophobic pentacyclic triterpane skeleton of BHP appears to be very limited. Unsaturation ($\Delta 6$ and/or $\Delta 11$) is rare, but supplementary methyl substituents at the 2 α , 2 β and 3 β positions of ring-A have been reported often and may indicate metabolism in some bacteria. Methylation at C3 has been reported for a variety of methanotrophic, methylotrophic and acetic bacteria^{9,10}. Methylation at C2 is common in cyanobacteria^{9,11}, as shown in Table 1, although it is not exclusive to this group. Of those other bacteria that have been studied, 2-methylhopanoids have also been found in pink pigmented facultative methylotrophs (PPFMs) related to *Methylobacterium organophilum*¹² and some nitrogen-fixing bacteria

Table 1 Abundances ($\mu\text{g per g dry wt}$) of hopanols analysed in cultured cyanobacteria, a prochlorophyte and various environmental samples

Cyanobacterial cultures and environmental samples analysed in this study	C31 5	2-MeC31 6	C32 7	2-MeC32 8
<i>Phormidium luridum</i> UTEX 426 (CCAP 1462/2)			540	74
<i>Chlorogloeopsis fritschii</i> ATCC 27193			65	7
<i>Synechococcus lividus</i> ATCC 27180			155	95
<i>Synechococcus</i> ATCC 29534 (<i>Aphanothece halophitica</i>)			20	30
<i>Synechococcus</i> ATCC 27144 (<i>Anacystis nidulans</i>)	39	63	25	23
<i>Gloeobacter violaceus</i> ATCC 29082			450	650
<i>Anabaena cylindrica</i> ATCC 27899			68	
<i>Fischerella</i> sp. ATCC 29538			2	
<i>Phormidium</i> sp. 'OSS4' Octopus Spring, YNP	179	258	<1	23
<i>Phormidium</i> sp. 'RCG' Rabbit Creek, YNP			161	
<i>Phormidium</i> sp. 'RCO' Rabbit Creek, YNP			<1	
<i>Phormidium</i> sp. 'OSS3' Octopus Spring, YNP			45	
<i>Chlorogloeopsis</i> sp. 'LA' Norris Geyser Basin, YNP	286		374	
<i>Calothrix</i> sp. 'TSB' Twin Springs, YNP			255	
<i>Oscillatoria limnetica</i> (Y. Cohen), Solar Lake, Israel			40	
<i>Oscillatoria</i> sp. 'GN' (E. Munoz), Guerrero Negro, Mexico			<1	
<i>Phormidium</i> sp. 'GN' (E. Munoz), Guerrero Negro, Mexico			<1	
<i>Oscillatoria</i> sp. (L. Stal) Mellum Island, Germany			27	
<i>Oscillatoria</i> sp. 10mfx (J. Bauld) Shark Bay, Australia			18	
Octopus Spring <i>Synechococcus-Chloroflexus</i> 60 °C mat*	14	20	23	
Octopus Spring <i>Synechococcus-Chloroflexus</i> 55 °C mat* ¹⁶	1	1	8	
Octopus Spring <i>Phormidium-Chloroflexus</i> 45 °C mat*	61	64	90	29
Fountain Paint Pot <i>Phormidium-Chloroflexus</i> 45 °C mat*	9	30	24	13
Rabbit Creek <i>Phormidium-Chloroflexus</i> 40 °C mat*	2	32	24	7
Shark Bay 'Smooth' Mat (J88/1 predom. <i>Microcoleus</i> sp.)†			1.2	
Shark Bay 'Tufted' Mat (J88/9 predom. <i>Lyngbya</i> sp.)†			0.7	
Shark Bay 'Smooth' Mat (J88/10 predom. <i>Microcoleus</i> sp.)†			0.7	
Shark Bay 'Tufted' Mat (J88/14 predom. <i>Lyngbya</i> sp.)†			20	
Shark Bay 'Pustular' Mat (J88/17 predom. <i>Entophysalis</i> sp.)†			1.0	
Data extracted from literature reports				
<i>Prochlorothrix hollandica</i> whole cells ¹¹			60	90
<i>Prochlorothrix hollandica</i> cell wall ¹¹			400	600
<i>Prochlorothrix hollandica</i> thylakoids ¹¹			940	1410
<i>Nostoc</i> sp. ATCC 27985 ⁸			250	
<i>Nostoc muscorum</i> B-1453-12b¶ ²⁶			3x	7x
<i>Nostoc</i> sp. PCC 6720 ²⁷	55	220	66	160
<i>Scytonema</i> sp. ATCC 29171 ⁸			100	
<i>Synechococcus</i> sp. PCC 6907 ²⁸			135	72
<i>Calothrix anomala</i> ¹⁵	15		<1	400
<i>Schizothrix</i> sp. ¹⁵	2		130	<1
<i>Anacystis montana</i> ¹⁵	<1		44	180
<i>Oscillatoria rubescens</i> ¹⁵	<1		430	<1
<i>Gloeobacter</i> sp. ¹⁵	<1		<1	2700
Various species (9)‡			3-140	
Various species (3)‡ ⁹	<1		<1	

* Indicates relative amounts of bacteriohopanol in mats and are for top photic zone (~1 mm for *Synechococcus* and up to 1 cm for *Phormidium*) and do not account for high levels of silica associated with dry weight.

† Indicates data do not account for high levels of CaCO₃ associated with dry weight.

|| Indicates an x-methylhopanol was detected but the identity and proportion are unspecified.

¶ Indicates only compound ratios and not absolute abundances available.

Indicates that a 2-methyl-C₃₀ hopanol precursor was also reported.

‡ Indicates that the original 1984 methodology was used and this may have underestimated BHP contents (M. Rohmer, personal communication).

of the genera *Azotobacter* and *Beijerinckia*¹³. However, the predominant 2-methylhopanoids in these organisms are diplopterols and diploptenes, which are C₃₁ alcohols and hydrocarbons. Only trace amounts of 2-methyl-BHP have been identified in *M. organophilum*¹⁴. This has significant implications for their preservation potential, because diplopterols readily dehydrate to diploptenes, which have very limited capacity to be incorporated into kerogen. BHP and 2-methyl-BHP, on the other hand, have multiple functional groups through which they can be incorporated into the kerogen matrix and preferentially preserved. Although 2-methyl-BHP may be found in unstudied taxa in the future, the cyanobacterial lineage currently comprises the only known quantitatively significant source^{9,11,15,16} with a conduit to selective preservation.

The C₃₂ hopanol (alcohol precursor of type 7 in Fig. 1) is by far the most common BHP derivative encountered in cyanobacteria. Its BHP precursors are probably the source of the C₃₂ carboxylic acid and C₃₁ hydrocarbon that are the most widespread geohopanooids in recent sediments. Around 43% of the listed samples contain 2-methyl analogues (6 and 8). Four out of five mats from the Yellowstone National Park hydrothermal environment contained significant 2-methyl-BHP, although none of five samples from the hypersaline environments of Shark Bay did. Although the present sample set gives limited coverage of modern environments, species

that have been cultured include one prochlorophyte and span a taxonomically diverse spectrum of cyanobacteria. The Yellowstone *Phormidium* conophyton mats that we studied all had abundant 2-methyl-BHP. These are the possible modern analogues for the conophyton stromatolites that have a sedimentary record reaching back 2,700 Myr (ref. 2). However, note that biomarker probes for the antiquity of cyanobacteria are unidirectional, as 2-methyl-BHP are not found in all cultured species nor all mats.

The sedimentary conversion of BHP to the C₂₇-C₃₅ geohopane series (3 in Fig. 1) has been extensively studied in connection with the application of these biomarkers to petroleum exploration⁷. The burial fate of 2-methyl-BHP is virtually identical to that of BHP except that the 2 β -methyl configuration of the original biochemicals (2 in Fig. 1) is progressively converted to the more thermodynamically stable 2 α -methyl configuration (4 in Fig. 1) in the 2-methyl geohopane series¹⁷. Despite this, we need to be cautious in how we validate fossil hydrocarbons. Establishing that they are indigenous to the host rock requires close attention to a variety of factors, as these compounds are mobile. Low levels of metamorphism, high contents of associated kerogen, covariance of the $\delta^{13}\text{C}$ of kerogen and bitumen, remoteness from younger oil-prone rocks and distinctive compound assemblages indicate syngeneity, especially when there is convergence of multiple lines of evidence for a particular sample. To the best of our knowledge, all

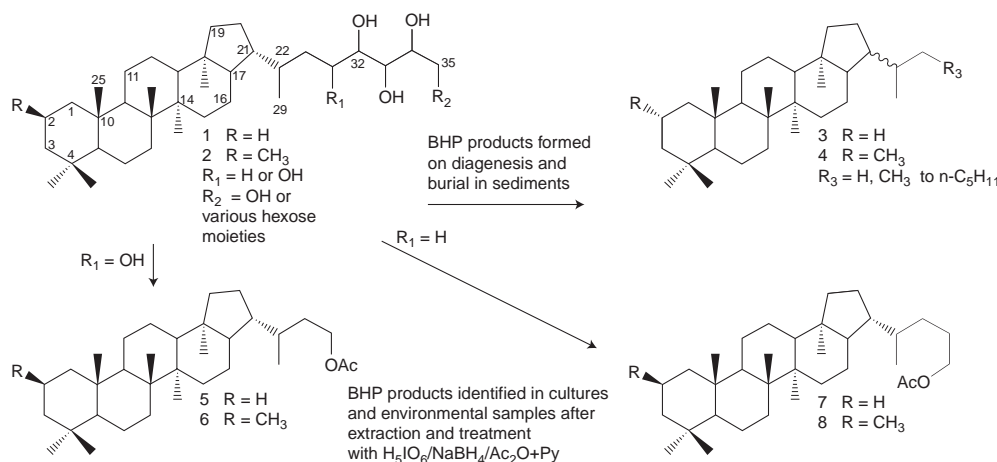


Figure 1 Structures of bacteriohopanepolyols (1, 2) typical of those found in cyanobacteria and their geohopane analogues recognized in sediments and

petroleum (3, 4). BHP from cultures and environmental samples were chemically reduced to hopanol acetates of types 5–8 and analysed by GC-MS.

the bitumens listed in Table 2 are indigenous to their host formations, whereas all the oils are derived from specific source rocks of the designated ages.

The data shown in Table 2 confirm earlier observations^{17,18} that higher relative abundances of 2-methylhopanes, expressed here as a 2-methylhopane index, are a distinctive feature of oils and bitumens derived from carbonate rocks. Moreover, there are high relative abundances of 2-methylhopanes in Proterozoic (>550 Myr) sediments of all lithologies compared to their Phanerozoic (<550 Myr) counterparts. Our data indicate that burial temperature strongly affects the 2-methylhopane index, because all immature samples analysed had low values. This indicates that generation requires cracking from chemically-bound precursors and independently supports their origin from BHP rather than diplopterols or diploptenes. The data for mature samples confirm previous observations that oils from carbonate source rocks have the highest 2 α -methylhopane indices¹⁷. Such sediments frequently form in warm, low-

latitude, shallow-water continental shelves, coastal lagoons, and other restricted marine settings. They also form in playa and saline lakes, as exemplified by the samples from the Early Cambrian Observatory Hill Formation, which had the highest 2-methylhopane indices we have encountered so far. Modern settings of this type are noted for the development of taxonomically diverse cyanobacterial mats^{19,20} and moderate-to-high rates of organic matter accumulation²⁰. It is likely that this has been the case for at least the past 2,500 Myr. Given the occurrence of 2-methyl-BHP in a wide variety of cyanobacterial taxa and cyanobacterial mats, a high abundance of 2-methylhopanes is expected in carbonate-derived oils of any age. Organic-rich shales, on the other hand, form in more open and distal marine settings including shelves, slopes and deltas, prone to clastic sediment inputs. In modern and Phanerozoic settings such as these, algae are the prime autotrophs with sedimentary carbon supplemented with inputs from allochthonous terrestrial organic matter. Planktonic and picoplanktonic cyanobacteria may be responsible for significant primary productivity in the modern ocean and our data indicate that they would have been more significant in times past, particularly in the Proterozoic²¹. Oils and bitumens from Proterozoic shales have higher 2-methylhopane indices (5–18.7%) than the mature Phanerozoic shale samples that we analysed (0.3–9%).

Given that 2-methyl-BHP are prominent lipids in cyanobacteria and cyanobacteria-dominated environmental samples, it is evident that 2-methyl-BHP and their derivative 2 α -methylhopanes also comprise a molecular record for oxygenic photosynthesis, as this is their preferred physiology. The high abundance of derivative 2 α -methylhopanes in bitumens from the 2,500 Myr Mt McRae Shale of the Hamersley basin provides direct evidence for oxygenic photosynthesis at this time and supports microfossil and other geological evidence that the process predates this. The Hamersley basin samples are also significant for another reason. Their sediment extracts extend the molecular biomarker record to the base of the Palaeoproterozoic, and are 700 Myr older than the oldest known biomarkers²². The Mt McRae Shale meets most of the important syngeneity criteria, especially with respect to having high organic carbon contents (0.5–6.9%) and hydrocarbon stereoisomer patterns that correlate well with other estimates of their thermal history (Ro \approx 1.5–1.9). The Pilbara craton of Western Australia, location of the Hamersley basin, is also remote from any known Phanerozoic petroleum sources. Although it is difficult to find suitably preserved Archaean (>2,500 Myr) samples, recent work on fluorescing, fluid-bearing inclusions in quartz crystals in sandstones of this age, and older, indicate that some hydrocarbons generated at this time may have been preserved²³ and are likely to

Table 2 2-Methylhopane indices for well characterized oils and sedimentary bitumens

Formation	Lithology	Age (Myr)	Index
Mt. McRae Shale (3)	Shale	~2,500	12.5
Wologorang Fm. (2)	Shale	~1,730	12.0
Barney Creek Fm. (6)	Shale	~1,640	8.5
Balbirini Fm.	Shale	~1,600	9.0
Yalco Fm. (1)	Shale	~1,485	14.7
McMinn Fm. (1)	Shale	~1,400	5.8
Nonesuch Fm. (2)	Shale	~1,000	4.7
Kwagunt Fm. (2)	Marl	~850	10.9
Kwagunt Fm. (1)	Shale	~850	18.7
Bitter Springs Fm (3)	Marl	~830	6.6
Draken Fm. (2)	Carbonate	~720	8.8
Ungoolya Fm. (4)	Shale	~560	6.7
Pertatataka Fm. (3)	Shale	~560	4.9
Shuram Fm. (1)	Marl	~560	12.7
Athel Fm. (1)	Marl	~550	11.2
Huqf Group oil (2)	Carbonate	~550	10.9
Observatory Hills Fm. (4)	Carb./evap.	530–540	32.4
Ouldburra Fm. (4)	Carbonate	530–540	9.9
Inca Fm.* (1)	Shale	505–510	2.4
Horn Valley Fm. (1)	Shale	460–485	5.3
Milligans Fm. (2)	Shale	330–345	9.4
Phosphoria Fm. oil (1)	Shale	270–300	0.5
Cooper basin oils (4)	Coal	250–270	4.0
Bowen basin oils (12)	Coal	250–270	8.9
Vulcan sub-basin oil (2)	Shale	150–160	4.5
Barrow sub-basin oil (2)	Shale	150–160	4.5
North Sea oil (1)	Shale	150–160	2.5
Julia Ck. Fm.* (1)	Shale	97–108	0.6
Taranaki basin (1)	Shale	61–65	0.3
Monterey Fm.* (2)	Carbonate	17–24	5.0
Monterey Fm.* (1)	Shale	6–8	0.9

* Samples above the oil window (see Methods).

hold a valid chemical fossil record²⁴.

Cyanobacterial BHP and 2-methyl-BHP may have oceanographic and palaeo-oceanographic applications as well, particularly with respect to evaluation of cyanobacterial primary productivity and their importance for the marine nitrogen and carbon cycles²⁵. As biomarkers such as BHP and chlorophylls carry ¹³C and ¹⁵N signatures¹⁶, their usefulness as tracers in modern aquatic and marine environments is significantly broadened. □

Methods

Total lipid extracts from cultures and environmental samples were analysed using a procedure modified and improved after ref. 8. Periodic acid oxidation followed by NaBH₄ reduction converted BHP to simpler hopanols amenable to purification by thin-layer chromatography and GC-MS analysis as acetate derivatives. BHP lacking a gem-diol function evade detection. The total number of culture samples with suitable data was 42 and, of these, only 6 failed to yield detectable hopanol. The data in Table 1 are from this study or directly extracted or calculated from published quantitative data.

The data for fossil hopanoids shown in Table 2 was derived from GC-MS-MS analyses⁵ and based on the *m/z* 412 → 191 transition for αβ-hopane (3 in Fig. 1, R₃ = H) and *m/z* 426 → 205 for its 2α-methyl analogue (4, R₃ = H). These are expressed as the 2-methylhopane index = % 4/4+3. Accompanying C₂₈-C₃₆ homohopanes (R = CH₃, R₃ = CH₃ to C₅H₇) were examined using the *m/z* 205 ion chromatograms and confirmed the relationships expressed here for the C₃₀ and 2α-methyl C₃₁ species. Age and lithology assignments for Phanerozoic samples were derived from AGSO databases. Assignments for Proterozoic sediments are primarily based on ref. 5, updated where possible. Numbers in parentheses indicate (*n*) samples of each rock unit. All samples are mature for oil generation except those marked with asterisks, which are above the oil window. Low 2α-methylhopane indices in these samples indicates incomplete release by thermal cracking of C₃₅ and 2-Me C₃₆ moieties bound in kerogen.

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Spatial scaling laws yield a synthetic theory of biodiversity

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Ecologists still search for common principles that predict well-known responses of biological diversity to different factors^{1–4}. Such factors include the number of available niches in space^{5–7}, productivity^{8–10}, area¹⁰, species' body size^{11–14} and habitat fragmentation. Here we show that all these patterns can arise from simple constraints on how organisms acquire resources in space. We use spatial scaling laws to describe how species of different sizes find food in patches of varying size and resource concentration. We then derive a mathematical rule for the minimum similarity in size of species that share these resources. This packing rule yields a theory of species diversity that predicts relations between diversity and productivity more effectively than previous models^{8–10}. Size and diversity patterns for locally coexisting East African grazing mammals and North American savanna plants strongly support these predictions. The theory also predicts relations between diversity and area and between diversity and habitat fragmentation. Thus, spatial scaling laws provide potentially unifying first principles that may explain many important patterns of species diversity.

The search for a 'unified' theory of diversity^{1–5} has focused on the premise that more species can exist within a habitat whenever they can more finely divide up space and different-sized resource 'packages'. Such partitioning may be constrained by the different body sizes of species^{5,7,11,12–14}, but the mechanisms by which organism size, resource availability and spatial structure of habitats control species diversity remain unclear^{1,2,7,11,14}. Here we employ spatial scaling laws to describe how species with different body sizes find resources in space, and how limits to the similarity in body size between any two species predicts the potential number of species in a community.

Individual organisms must search within a space of suitable physical/chemical conditions (habitat) to find resources, which are often only available inside other material (food) (Fig. 1). Therefore, resources available to organisms are nested within food, and available food is nested within habitat. For example, insect herbivores move through suitable microclimates on terres-