Normal is passé; extreme is chic. While Aristotle cautioned "everything in moderation", the Romans, known for their excesses, coined the word 'extremus', the superlative of exter ("being on the outside"). By the fifteenth century 'extreme' had arrived, via Middle French, to English. At the dawn of the twenty-first century we know that the Solar System, and even Earth, contain environmental extremes unimaginable to the 'ancients' of the nineteenth century. Equally marvellous is the detection of organisms that thrive in extreme environments. Macelroy\(^1\) named these lovers ("philos" to the Greeks) of extreme environments 'extremophiles'.

The discovery of extreme environments and the organisms that inhabit them has made more plausible the search for life outside the Earth, and even the possibility of panspermia (the transport of life from one planet to another). The discovery of extremophiles has also put vitality into the biotech industry and dreams of stock options in the future. Each recent report of liquid water existing elsewhere in the Solar System has reverberated through the international press and excited the imagination of humankind. Why? Because in the past few decades we have come to realize that where there is liquid water on Earth, virtually no matter what the physical conditions, there is life. What we previously thought of as insurmountable physical and chemical barriers to life, we now see as yet another niche harbouring 'extremophiles'. This realization, coupled with new data on the survival of microbes in the space environment and modelling of the potential for transfer of life between celestial bodies, suggests that life could be more common than previously thought. Here we examine critically what it means to be an extremophile, and the implications of this for evolution, biotechnology and especially the search for life in the Universe.

What is an extremophile?

An organism that thrives in an extreme environment is an extremophile; in more than one extreme it is a polyextremophile. Examples of the latter would include \textit{Sulfolobus acidocaldarius}, an archaea that flourishes at pH 3 and 80°C (Fig. 1). 'Extremes' include physical extremes (for example, temperature, radiation or pressure) and geochemical extremes (for example, desiccation, salinity, pH, oxygen species or redox potential) (Table 1). It could be argued that extremophiles should include organisms thriving in biological extremes (for example, nutritional extremes, and extremes of population density, parasites, prey, and so on).

'Extremophile' conjures up images of prokaryotes, yet the taxonomic range spans all three domains. Although all hyperthermophiles are members of the Archaea and Bacteria, eu karyotes are common among the psychrophiles, acidophiles, alkaliphiles, piezophiles, xerophiles and halophiles (which respectively thrive at low temperatures, low pH, high pH, and under extremes of pressure, desiccation and salinity; see http://www.astrobiology.com/ extreme.html for an overview). Extremophiles include multicellular organisms, and psychrophiles include vertebrates.

Although these characterizations seem straightforward, three philosophical issues need further exploration. First, what is 'extreme'? Perhaps 'extreme' is in the eyes of the beholder. It is clear that to a thermophile that dies at 21°C and a piezophile that finds atmospheric pressure 'extreme', what determines an extremophily is based on definitions that are perhaps anthropocentric. There are two possibilities that are more scientifically tenable. The first is based on an evolutionary perspective — that is, the earliest...
environment for life defines what is ‘normal’. If life arose in a high-temperature, anoxic hydrothermal vent, any environment that deviates from that is ‘extreme’. The second, which we favour, is based on a more objective, physical definition of ‘extreme’. This definition is congruent with the colloquial definition, with exceptions. All physical factors are on a continuum, and extremes in the conditions that make it difficult for organisms to function are ‘extreme’. For example, to maintain chemistry in an aqueous environment, cells need certain temperatures, pH and solutes, precise control over biomolecules and electric currents, and the ability to repair damage. There are certain conditions that will destroy biomolecules, such as desiccation, radiation and oxygen. Regarding the last of these conditions, there are certain extremes than the vegetative forms. Trees, frogs, insects and fish can tolerate remarkably low temperatures during the winter as a result of seasonal shifts in physiology. Tardigrades (‘water bears’) in the tun state, can survive temperatures from −253 °C to 151 °C, X-rays, vacuum and, when in perfluorocarbon, pressures up to 600 MPa, almost 6,000 times atmospheric pressure at sea level.

Environmental extremes

Liquid water is the sine qua non of life on Earth, and arguably any life in our Solar System. Furthermore, life requires an input of energy, but must also be able to control energy flow. Redox chemistry is universal. As life is based on organic chemistry, such chemistry must be allowed to operate. An extremophile must either live within these parameters, or guard against the outside world in order to maintain these conditions intracellularly. With these rules in mind, we examine selected environmental parameters, summarized in Table 1.

Temperature

Temperature creates a series of challenges, from the structural devastation wrought by ice crystals at one extreme, to the denaturation of biomolecules at the other. The solubility of gases in water is correlated with temperature, creating problems at high temperature for aquatic organisms requiring O₂ or CO₂. Temperatures approaching 100 °C normally denature proteins and nucleic acids, and increase the fluidity of membranes to lethal levels. Chlorophyll degrades above 75 °C, excluding photosynthesis (Fig. 2).

Yet, in nature thermal preferences range from hyperthermophilic (maximum growth >80 °C) to psychrophilic (maximum growth <15 °C). The most hyperthermophilic organisms are archaea, with Pyrolobus fumarii (Crenarchaeota), a nitrat-
Temperature limits for life. The highest and lowest temperature for each major taxon is given. Archaia are in red, bacteria in blue, algae in light green, fungi in brown, protozoa in yellow, plants in dark green and animals in purple.

Reducing chemolithoautotroph, capable of growing at the highest temperatures of up to 113 °C (ref. 15). Hyperthermophile enzymes can have an even higher temperature optimum; for example, activity up to 142 °C for amylopullulanase24. There are thermophiles among the phototrophic bacteria (cyanobacteria, purple and green bacteria), eubacteria (Bacillus, Clostridium, Thiothrix, Desulfo-
tomaculum, Thermus, lactic acid bacteria, actinomycetes, spirochetes and numerous other genera) and the archaea (Pyrococcus, Thermo-
coccus, Thermoplasma, Sulfolobus and the methanogens). In contrast, the upper limit for eukaryotes is ~60 °C, a temperature suitable for some protozoa, algae and fungi. The maximum temperature for mosses is lower by another 10 °C, for vascular plants it is about 48 °C, and for fish it is 40 °C, possibly owing to the low solubility of oxygen at high temperatures (Fig. 3).

Representatives of all major taxa inhabit temperatures just below 0 °C. Many microbes and cell lines can be preserved successfully at −196 °C (liquid nitrogen), but the lowest recorded temperature for active microbial communities is substantially higher, at −18 °C (ref. 17). Among animals, the Himalayan midge is active at −18 °C (ref. 18). Liquid water not only is a solvent for life as we know it, but also is important either as a reactant or product in most metabolic processes17. At low temperatures with nucleation, water freezes. The resulting ice crystals can rip cell membranes, and solution chemistry stops in the absence of liquid water. Freezing of intracellular water is almost invariably lethal. The only exception to this rule reported so far, outside of cryopreservation, is the nematode Panagrolaimus davidi, which can withstand freezing of all body water25.

Radiation
Radiation is energy in transit, either as particles (for example, neutrons, electrons, protons, alpha particles or heavy ions) or electromagnetic waves (for example, gamma rays, X-rays, ultraviolet (UV) radiation, visible light, infrared, microwaves or radio waves). Exceptional levels of radiation — sufficient to qualify for ‘extremophile’ status — rarely occur on the Earth naturally, but intense levels of UV and ionizing radiation are well-studied because of their importance to medicine, energy production, warfare and space travel. The dangers of UV and ionizing radiation range from decreased motility to inhibition of photosynthesis, but the most serious is damage to nucleic acids. Direct damage to DNA or indirect damage through the production of reactive oxygen species creates modified bases and single- and double-strand breaks.

The bacterium D. radiodurans is famous for its ability to withstand ionizing radiation (up to 20 kGy of gamma radiation) and UV radiation (doses up to 1,000 J m⁻²), but this extraordinary resistance is thought to be a by-product of resistance to extreme desiccation27. Other organisms that can stand high levels of radiation are two Rubrobacter species28 and the green alga Dunaliella bardawil29.

Pressure
Hominids evolved at an atmospheric pressure of 101 kPa (≈ 1 atmosphere = 1.013 bar), although our aquatic ancestors originated under hydrostatic pressure. Hydrostatic pressure increases at a rate of 10.5 kPa per metre depth, compared with 22.6 kPa per metre for lithostatic pressure. Pressure decreases with altitude, so that by 10 km above sea level, atmospheric pressure is almost a quarter of that at sea level. The boiling point of water increases with pressure, so water at the bottom of the ocean remains liquid at 400 °C. Because liquid water normally does not occur above ~100 °C, increased pressure can increase the optimal temperature for microbial growth, but usually by only a few degrees21.

Pressure challenges life because it forces volume changes. Pressure compresses packing of lipids resulting in decreased membrane fluidity25. If a chemical reaction results in an increase in volume, as most do, it will be inhibited by an increase in pressure26. Although many organisms have adapted to very high pressures, a sudden change can be lethal, an effect only too well known to divers.

The Mariana trench (11° 22’ N, 142° 25’ E) is the world’s deepest sea floor at 10,898 m, yet it harbours organisms that can grow at standard temperature and pressure. It also has yielded obligatory piezophilic species22 that can grow at 70 to 80 MPa, but not below 50 MPa.

One component of pressure is gravity. Until now, organisms on Earth have, except for brief moments, lived at 1g. Space exploration will include extended periods in locations with gravity regimes different from our own: for example, launch vehicles (variable g), the International Space Station (microgravity), the Moon (0.17g) and Mars (0.38g). Although most of the concern with the effect of gravity have focused on human health, gravitational effects also have been found for microbes and include changes in biomass production, an increase in conjugation and changes in membrane permeability in Escherichia coli25.

Desiccation
Water possesses many properties that seem to make it the essential solvent for life. It has high melting and boiling points with a wide temperature range over which it remains liquid, and a high dielectric
constant important for its solvent action. Water expands near its freezing point, and it forms hydrogen bonds. No other compound possesses all of these traits. Thus, water limitation is an extreme environment. Organisms that can tolerate extreme desiccation enter anhydrobiosis, a state characterized by little intracellular water and no metabolic activity. A variety of organisms can become anhydrobiotic, including bacteria, yeast, fungi, plants, insects, tardigrades, mycophagous nematodes and the shrimp Artemia salina\textsuperscript{39–41}.

Mechanisms of death due to anhydrobiosis include irreversible phase changes to lipids, proteins and nucleic acids such as denaturation and structural breakage through Maillard reactions, and accumulation of reactive oxygen species during drying, especially under solar radiation\textsuperscript{42–44}.

Salinity
Organisms live within a range of salinities, from essentially distilled water to saturated salt solutions. Osmophily refers to the osmotic aspects of life at high salt concentrations, especially turgor pressure, cellular dehydration and desiccation. Halophily refers to the ionic requirements for life at high salt concentrations. Although these phenomena are physiologically distinct, they are environmentally linked. Thus, a halophile must cope with osmotic stress. Halophiles include a range of microbes, but some archaea, cyanobacteria and the green alga Dunaliella salina can withstand periods in saturated NaCl.

\textbf{pH}
\textit{pH} is defined as \(- \log_{10}[\text{H}^+]\). Biological processes tend to occur towards the middle range of the \textit{pH} spectrum, and intracellular and environmental \textit{pH} often fall in this range (for example, the \textit{pH} of sea water is \(-8.2\)). However, in principle, \textit{pH} can be high, such as in soda lakes or mental \textit{pH} often fall in this range (for example, the \textit{pH} of sea water is \(-7.1\)). The red alga Cephalosporium cerebriae and Picrophilus torridus have been isolated from \textit{pH} 0 in acid mine drainage in Iron Japanese soils permeated with solfataric gases, and had optimal growth at \textit{pH} 0.7 and 60°C (ref. 39). Archaea have also been found flourishing under extreme acidity. The aerobic heterotrophs Picrophilus oshimae and Picrophilus torridus were isolated from Japanese soils permeated with solfataric gases, and had optimal growth at \textit{pH} 0.7 and 60°C (ref. 40). Ferroplasma acidarmanus has been described growing at \textit{pH} 0 in acid mine drainage in Iron Mountain in California\textsuperscript{39}, thriving in a brew of sulphuric acid and high levels of copper, arsenic, cadmium and zinc with only a cell membrane and no cell wall.

Alkaliphiles prefer high \textit{pH}, which is an equally challenging environment. As with low \textit{pH}, there is often a difference of 2 or more \textit{pH} units between the internal and external milieu of the cell. Protons are scarce, creating energetic hurdles for aerobic prokaryotes with a membrane-bound ATP synthase\textsuperscript{45}. Representatives of all domains and kingdoms of eukaryotes are able to tolerate \textit{pH} as high as \(-11\) (Fig. 4; refs 43, 44).

\textbf{Oxygen}
The Earth has been anaerobic throughout most of the history of life. Today organisms inhabit environments ranging from strictly anaerobic to aerobic. Aerobic metabolism is far more efficient than anaerobic, but the exploitation of oxygen metabolism has its costs. Oxidative damage resulting from the reduced forms of molecular oxygen, especially the hydroxyl radical, is extremely serious. Oxidative damage has been implicated in an array of health problems from ageing\textsuperscript{47} to cancer\textsuperscript{48}, and has a range of consequences in nature (L.J.R., C.L. Wilson, N. Chough and R. I. Donaldson, unpublished results).

Reactive oxygen species are a pervasive threat. There is photochemical production of such species as H$_2$O$_2$ by UVA radiation (320–400 nm) within cells\textsuperscript{7}, and metabolic production during aerobic metabolism and photosynthesis\textsuperscript{2}. Other endogenous sources of reactive oxygen species in eukaryotes include mitochondrial respiration (a significant source of O$_2^*$), cytochrome P450 metabolism of hydroperoxides (an important source of O$_2^*$ (singlet oxygen)), production of uric acid, and oxidative bursts used in fighting pathogens in animals and plants. Exogenous sources include the photochemical production of H$_2$O$_2$ in aquatic systems\textsuperscript{2}, and the production of the hydroxyl radical by ionizing radiation. The presence of oxygen can enhance radiation-induced DNA damage\textsuperscript{2}.

\textbf{Other extreme conditions}
A little creative thinking suggests other physical and chemical extremes not considered here. These include extremes in gas composition (\textit{Cyanidium} grow in media ventilated with pure CO$_2$ (ref. 48)), redox potential, toxic or xenobiotic (synthetic) compounds, and heavy metal concentration\textsuperscript{49}. There are organisms that can live immersed in high levels of organic solvents\textsuperscript{50}. The electric eel (\textit{Electrophorus electricus}) can produce, and thus must tolerate, strong electric currents.

\textbf{How do they do it?}
It is critical for an organism to maintain function, and the easiest approach to achieve this is to keep the external environment out. For example, \textit{Cyanidium caldarium} and \textit{Dunaliella acidophila} are found at \textit{pH} 0.5, yet have near neutral cytoplasm\textsuperscript{38,42}, although this implies that extracellular proteins are acid-tolerant. The next step is to remove the problem as fast as possible. Heavy metal-resistant bacteria use an efflux pump to remove, for example, zinc, copper and cobalt, but not mercury, which is volatilized\textsuperscript{49}. If it is impossible to keep the environment out, evolutionary responses entail protective mechanisms, altering physiology or enhancing repair capabilities. Research has focused so far on three key classes of biomolecules: nucleic acids, membrane lipids and proteins. For nucleic acids, function and structure are linked inextricably. DNA is especially vulnerable to high temperature, radiation, oxidative damage and...
desiccation. This can lead either to convergence or to multiple ways to solve the problem of living in a particular environment. Understanding the alternatives by using extremophiles on Earth as a sample should help us understand evolutionary processes on Earth, predict them elsewhere, and be useful in commercial exploitation of extremophiles.

High-temperature adaptation

High temperature increases the fluidity of membranes. To maintain optimal membrane fluidity the cell must adjust the composition of the membrane including the amount and type (for example, saturated versus unsaturated) of lipids. Temperature also affects the structure and function of proteins. Ways that proteins have evolved to cope with high temperatures include increasing ion-pair content, forming higher-order oligomers and decreasing flexibility at room temperature. Decreasing the length of surface loops is also known, in particular those loops that connect elements of secondary structure, optimize electrostatic and hydrophobic interactions, and exchange amino acids to increase internal hydrophobicity and helix propensity of residues in α-helices.

DNA at high (>70 °C) temperatures is subject normally to denaturation and chemical modification, yet the DNA of hyperthermophiles such as Pyrococcus furiosus is known to be more stable in vivo than that of a mesophile such as Escherichia coli. Monovalent and divalent salts enhance the stability of nucleic acids because these salts screen the negative charges of the phosphate groups, and because KCl and MgCl2 protect the DNA from depurination and hydrolysis.

The G–C pair of nucleic acids is more thermostable than the A–T pair or A–U pairs because of the additional hydrogen bond. But elevated G + C ratios are not found among thermophilic prokaryotes because of the stability of the chromosomal DNA, although thermostability is correlated with G + C content of their ribosomal and transfer RNAs.

Cold-temperature adaptation of protein occurs, although not always in ways that would be predicted from thermophile enzymes. At low temperatures there are low levels of free energy, so to decrease activation energy an enzyme must have a high degree of conformational complementarity with its substrate. At cold temperatures proteins become more rigid, implying that enhancing flexibility can restore function. Studies of α-amylase from the psychrophile Alteromonas haloplanktes, an enzyme with increased reliance of the active site to its substrate, activation energy an enzyme must have a high degree of conformational complementarity with its substrate.

Table 1 Classification and examples of extremophiles

<table>
<thead>
<tr>
<th>Environmental parameter</th>
<th>Type</th>
<th>Definition</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Hyperthermophile</td>
<td>Growth &gt;80°C</td>
<td>Pyrobacillus furiosus, 113 °C Pyrococcus furiosus, Synechococcus livids Homo sapiens Psychrobacter, some insects</td>
</tr>
<tr>
<td></td>
<td>Thermophile</td>
<td>Growth 60–80°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mesophile</td>
<td>15–60°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Psychrophile</td>
<td>&lt;15°C</td>
<td></td>
</tr>
<tr>
<td>Radiation</td>
<td>Barophile</td>
<td>Weight-loving</td>
<td>Demococcus radostanis, For microbe, 130 MPa</td>
</tr>
<tr>
<td></td>
<td>Plesophile</td>
<td>Pressure-loving</td>
<td></td>
</tr>
<tr>
<td>Gravity</td>
<td>Hypergravity</td>
<td>&gt;1g</td>
<td>None known</td>
</tr>
<tr>
<td></td>
<td>Hypogravity</td>
<td>&lt;1g</td>
<td>None known</td>
</tr>
<tr>
<td>Vacuum</td>
<td>Tolerates vacuum (space devoid of matter)</td>
<td>Tardigrades, insects, microbes, seeds</td>
<td></td>
</tr>
<tr>
<td>Desiccation</td>
<td>Xerophiles</td>
<td>Antihydrotic</td>
<td>Artemia salina, nematodes, microbes, fungi, lichens</td>
</tr>
<tr>
<td>Salinity</td>
<td>Halophile</td>
<td>Salt-loving (2–5 M NaCl)</td>
<td>Halobacteriaceae, Dunaliella salina</td>
</tr>
<tr>
<td>pH</td>
<td>Alkaliphile</td>
<td>pH &gt; 9</td>
<td>Natronobacterium, Bacillus firmus OF4, Spirulina spp. (pH 10.5) Cyanidium caldarium, Ferroplasma sp. (both pH 10.5)</td>
</tr>
<tr>
<td></td>
<td>Acidophile</td>
<td>low pH-loving</td>
<td></td>
</tr>
<tr>
<td>Chemical extremes</td>
<td>Anaerobe</td>
<td>Cannot tolerate O2</td>
<td>Methanococcus jannaschii Clostridium</td>
</tr>
<tr>
<td></td>
<td>Microaerophile</td>
<td>Tolerates some O2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aerobe</td>
<td>Requires O2</td>
<td>H. sapiens</td>
</tr>
<tr>
<td></td>
<td>Gases</td>
<td>Can tolerate high concentrations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Metals</td>
<td>of metal (metalotolerant)</td>
<td></td>
</tr>
</tbody>
</table>

Cold-temperature adaptation of protein occurs, although not always in ways that would be predicted from thermophile enzymes. At low temperatures there are low levels of free energy, so to decrease activation energy an enzyme must have a high degree of conformational complementarity with its substrate. At cold temperatures proteins become more rigid, implying that enhancing flexibility can restore function. Studies of α-amylase from the psychrophile Alteromonas haloplanktes, an enzyme with increased reliance of the active site to its substrate, activation energy an enzyme must have a high degree of conformational complementarity with its substrate.

Radiation and oxidative damage

Radiation and oxidative damage have always been common on Earth (L.J.R., C. L. Wilson, N. Chough and R. I. Donaldson, unpublished results). Mechanisms to avoid or repair environmentally encountered...
damage include production of antioxidants and detoxifying enzymes, avoidance behaviour and repair mechanisms. D. radiodurans copes with extraordinary radiation levels by containing a unique repair mechanism that involves reassembling of fragmented DNA.

Pressure
Pressure is known to alter gene expression. When pressure increases or temperature decreases, the molecules in lipid membranes pack tighter, resulting in decreased membrane fluidity. Often organisms circumvent this problem by increasing the proportion of unsaturated fatty acids in their membranes. Pressure can also help stabilize enzymes. High pressure can damage DNA and proteins in particular, so survival necessitates avoidance of damage or high repair rates.

Salinity and desiccation
Many microorganisms respond to increases in osmolarity by accumulating osmotica in their cytosol, which protects them from cytoplasmic dehydration and desiccation. With the exception of the Halobacteriaceae, which use K⁺ as their osmoticum, glycine betaine is the most effective osmoticum in most prokaryotes. Osmotic concentration increases during desiccation, so responses are similar to those of a cell in high-salt environments. Compatible solutes such as K⁺, glutamate, glutamine, proline, glycine betaine, sucrose and trehalose accumulate away from proteins, forcing water nearby and thus stabilizing them, and possibly stabilizing dry membranes. DNA damage is caused by increasing levels of desiccation from vacuum to pressure.

pH
Organisms that live at the extremes of pH are able to do so by maintaining their cytoplasm at the same pH as their mesophilic relatives, thus obviating the need for evolution of altered internal physiology. Active mechanisms to achieve this may involve secondary proton uptake mediated by membrane-associated antiporters. Passive mechanisms include negatively charged cell-wall polymers in alkaliphiles, and unusual bioenergetics, unusual permeability properties, positive surface charges, high internal buffer capacity, overexpression of H⁺ export enzymes and unique transporters for acidophiles.

Examples of extreme environment ecosystems
Hotsprings and geysers
Hotsprings and geysers are characterized by hot water and steam, and sometimes low pH and noxious elements such as mercury. The field was reviewed by Brock, and much recent work has been inspired by evolutionary biologists, biotechnology potential and astrobiology.

Deep sea
The deep-sea environment has high pressure and cold temperatures (1–2 °C), except in the vicinity of hydrothermal vents which are underwater geysers. In vents the temperature may be as high as 400 °C (ref. 74), but water remains liquid owing to the high hydrostatic pressure. Hydrothermal vents have a pH range from about 3 to 8 (ref. 75) and unusual chemistry. In 1977 the submarine Alvin found life 2.6 km deep along the East Pacific Rise, a centre of sea-floor spreading. Life forms range from microbes to invertebrates. Hydrothermal vents possibly were critical to evolution. Solution chemistry of hydrothermal vent systems is compatible with prebiotic chemistry leading to the origin of life (but see ref. 55). Phylogenetic evidence points to thermophiles as the last common ancestor. Either life arose in a vent, or only thermophiles were able to survive the last of the major impacts during the late bombardment period.

Hypersaline environments
Hypersaline environments include salt flats, evaporation ponds, natural lakes (for example, Great Salt Lake) and deep-sea hypersaline basins. These communities often are dominated by halophilic archaea, including square archaea, or D. salina. Other organisms are...
found at 25–33% salinity, including bacteria for example, *Ectothiorhodospira halochloris*, cyanobacteria (for example, *Aphanothecaceae halophytica*, *Phormidium* sp. and *Schizothrix arenaria*), green algae (for example, *D. salina* and *Asteromonas gracilis*), diatoms (for example, *Amphora coffeaeformis* and species of *Navicula* and *Nitzschia*) and protozoa (for example, *Blepharisma halophila* and species of *Bodo*, *Phyllophitus* and *Tetranites*). There are halophilic yeasts and other fungi, but they are not nearly as halophilic as other microbial taxa.

**Evaporites**

Evaporite deposits consisting primarily of halite (NaCl), gypsum (CaSO₄·2H₂O) or anhydrite (CaSO₄) and containing bacterial and algal assemblages are well known in the fossil record and are still geographically widespread. Norton and Grant showed that microorganisms entrapped in fluid inclusions of growing NaCl crystals may be motile for three weeks, and may remain viable for up to six months. Rothschild and colleagues demonstrated that microorganisms inhabiting gypsum halite crusts perform carbon and nitrogen fixation while inside the dry crystals of the crust for at least a year. Although highly controversial, bacteria might survive for millions of years in the fluid inclusions of salt deposits including evaporites.

**Deserts**

Deserts are extremely dry, and cold or hot. Water is always a limiting factor, so such ecosystems are often dominated by microbiotic crusts. The Atacama Desert is one of the oldest, driest hot deserts on Earth. The coldest, driest places on Earth are the dry valleys of Antarctica. The primary inhabitants for both hot and cold deserts are cyanobacteria, algae and fungi that live a few millimetres beneath the sandstone rock surface. Although the endolithic communities in the Antarctic desert are based on photosynthesis (cyanobacteria, lichens and green algae), these microbes have adapted to long periods of darkness and dry conditions interspersed with dustings of dry snow, that upon melting are brief sources of water. Second, some ice environments such as permafrost contain "a community of survivors." It is unlikely that the inhabitants of such an environment actually prefer this environment, rather they have found themselves trapped in the ice and are more resistant than others that have suffered as similar fate. Microbial communities in sea ice contain algae (mostly diatoms), protozoa, bacteria and some archaea.

**Ice, permafrost and snow**

From high-altitude glaciers coloured pink with ‘watermelon’ or ‘blood’ snow (often green algae with photoprotective secondary carotenoids) to the polar permafrost, microbial life has used frozen water as a habitat. But two caveats should be noted. First, some ice contains liquid brine inclusions that provide the actual habitat for water as a habitat. But two caveats should be noted. First, some ice environments such as permafrost contain "a community of survivors." It is unlikely that the inhabitants of such an environment actually prefer this environment, rather they have found themselves trapped in the ice and are more resistant than others that have suffered as similar fate. Microbial communities in sea ice contain algae (mostly diatoms), protozoa, bacteria and some archaea.

**Atmospheres**

The ability of an organism to survive in the atmosphere is a function of its ability to withstand desiccation and exposure to UV radiation. An airborne biota exists, although it is unclear whether it constitutes a functional ecosystem or is merely a live, but inactive, aerial suspension of organisms and their spore forms. Airborne organisms may travel across the Earth for hundreds to thousands of kilometres, and several kilometres up into the atmosphere. We argue that this field of aerobiology is critical to the enterprise of looking for life elsewhere in the Universe and further that it could be important in panspermia. In our view, it is one of the last frontiers of biological exploration on Earth, a view supported by the recent suggestion that life could have arisen in aerosols. On the present-day Earth, aerosols contain up to 50% organic material, and can acquire a lipid coating from the water below, meteorite-derived iron and nickel from the stratosphere, and energy from solar radiation — conditions conducive to the origin of life.

**Space: new categories of extreme environments**

Flight technology has enabled biological studies of space. Four main environments are currently of interest: manned-flight vehicles, interplanetary space (because of the potential for panspermia), and the planet Mars and jovian moon Europa (because of the possibility of liquid water and thus life) (Table 2). Thus, it is urgent that we define the environmental envelope for life, as well as conditions conducive to the origin of life, from hydrothermal to atmospherically to hypersaline parameters.

**Mars**

Mars is, for the most part, frigid (for current temperature, see http://emma.la.asu.edu/daily.html). The atmosphere receives 43% as much radiation as Earth, but attenuation through the thin, weak atmosphere is, for the most part, frigid (for current temperature, see http://emma.la.asu.edu/daily.html). The atmosphere receives 43% as much radiation as Earth, but attenuation through the thin, weak atmosphere is, for the most part, frigid (for current temperature, see http://emma.la.asu.edu/daily.html). The atmosphere receives 43% as much radiation as Earth, but attenuation through the thin, weak atmosphere is, for the most part, frigid (for current temperature, see http://emma.la.asu.edu/daily.html). The atmosphere receives 43% as much radiation as Earth, but attenuation through the thin, weak atmosphere is.
**Table 3 Examples of extremophiles in industry and biotechnology**

<table>
<thead>
<tr>
<th>Industrial process</th>
<th>Biomolecule</th>
<th>Advantages</th>
<th>Source organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis of starch to produce soluble</td>
<td>α-Amylase</td>
<td>High stability, acidic, bacterial amylase</td>
<td><em>Bacillus stearothermophilus</em></td>
</tr>
<tr>
<td>detergents, maltodextrins and corn</td>
<td></td>
<td></td>
<td>G-ZYME G-995</td>
</tr>
<tr>
<td>syrups</td>
<td></td>
<td></td>
<td>(Enzyme Bio-System Ltd)</td>
</tr>
<tr>
<td>Paper bleaching</td>
<td>Xylanases</td>
<td>Decreases amount of bleach needed</td>
<td>Thermophiles</td>
</tr>
<tr>
<td>Prevent stalling in range of baked</td>
<td>α-Amylase</td>
<td>Gives boost to yeast fermentation</td>
<td>Highest-stability bacterial amylase</td>
</tr>
<tr>
<td>products</td>
<td></td>
<td></td>
<td>available, G-ZYME G-995</td>
</tr>
<tr>
<td>Food processing, baking, brewing,</td>
<td>Proteases</td>
<td>Stable at high temperatures</td>
<td>Thermophiles</td>
</tr>
<tr>
<td>detergents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR reaction</td>
<td>DNA polymerase</td>
<td>No need to add additional enzyme</td>
<td>Thermophiles</td>
</tr>
<tr>
<td>Cheese maturation, dairy production</td>
<td>Neutral proteases</td>
<td>Stable at low temperatures</td>
<td>Psychrophiles</td>
</tr>
<tr>
<td>Degradation of polymers in detergents</td>
<td>Proteases, amyloses, lipases</td>
<td>Improved performance of detergent</td>
<td>Psychrophiles</td>
</tr>
<tr>
<td>Degradation of polymers in detergents</td>
<td>Cellulases, proteases, amyloses, lipases</td>
<td>Stable at high pH</td>
<td>Alkaliphiles</td>
</tr>
<tr>
<td>Mariculture</td>
<td>Polyunsaturated fatty acids</td>
<td>Produced in cold temperatures</td>
<td>Psychrophiles</td>
</tr>
<tr>
<td>Bioremediation</td>
<td>Reduction of oil spills</td>
<td>Works efficiently in cold waters</td>
<td>Psychrophiles</td>
</tr>
<tr>
<td>Pharmaceuticals</td>
<td>Polyunsaturated fatty acids</td>
<td></td>
<td>Psychrophiles</td>
</tr>
<tr>
<td>Biosensors</td>
<td>Dethydrogenases</td>
<td></td>
<td>Psychrophiles</td>
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<tr>
<td>Desulphonisation of coal</td>
<td>Sulphur oxidation</td>
<td></td>
<td>Acidiphiles</td>
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<td>Antibiotic production</td>
<td>Antibiotics</td>
<td></td>
<td>Alkaliphiles</td>
</tr>
<tr>
<td>Food colouring</td>
<td>Curcumin</td>
<td>Inexpensive to produce</td>
<td>Halophiles/Dunaliella</td>
</tr>
<tr>
<td>Pharmaceuticals</td>
<td>Glycerol, compatible solutes</td>
<td>Inexpensive to produce</td>
<td>Halophiles</td>
</tr>
<tr>
<td>Surfactants for pharmaceuticals</td>
<td>Membranes</td>
<td></td>
<td>Halophiles</td>
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**CO₂-rich atmosphere is minimal, resulting in high surface fluxes of radiation >200 W m⁻². Surface oxidants degrade organic carbon on the surface, which explains the negative results of the 1976 Viking missions. The atmospheric pressure is low (0.6–0.8 kPa), so liquid water is unstable on the surface, although hydrogeological evidence from the Mars Global Surveyor hints that liquid water may even flow today under the surface. Attention is now focused on the possibility of a subsurface biota, similar to the deep subsurface or hydrothermal communities found on Earth.**

Could life survive on the extreme harsh conditions of the martian surface? There are terrestrial organisms that hypothetically could withstand one or more of the martian extremes, but they would need protection. Mancinelli and Klovstad demonstrated that a monolayer of *B. subtilis* spores protected by a 10-μm-thick dust layer can survive UV exposure for weeks and probably years when exposed to a simulated martian UV-radiation flux. Thus, certain terrestrial microbes might survive on Mars.

**Europa**

Jupiter’s moon Europa may harbour a subsurface water ocean. This putative ocean lies beneath an ice layer too thick to allow photosynthesis. However, Chyba has hypothesized that disequilibrium chemistry in the ocean’s ice cover, driven by charged particles accelerated in Jupiter’s magnetosphere, could produce sufficient organic and oxidant molecules for a europa biosphere. Lake Vostok in Antarctica possesses a perennially thick (3 km) ice-cover that precludes photosynthesis below, thus making it a good model system for determining how a potential europa biosphere might survive.

**The space environment**

The theory of panspermia, as proposed by Richter, Lord Kelvin and Arrhenius, holds that reproductive bodies of living organisms can exist throughout the Universe and develop wherever the environment is favourable. This implies that conditions favourable to the development of life prevailed at different locations in the Universe and at different times. Major criticisms of panspermia are that living organisms will not survive long exposure to space, and that it avoids the issue of where life began. But results of the Long Duration Exposure Facility and BioPan space experiments, which showed that microbes can survive in space, as well as the fact that organic compounds have been found in meteorites, has led to a re-examination of the feasibility of interplanetary transfer of living material, particularly microbes.

Space is extremely cold, subject to unfiltered solar radiation, solar wind, galactic radiation, space vacuum and negligible gravity. At the distance of the Earth from the Sun, solar irradiance is 1,360 W m⁻². Of this, 45% is infrared light, 48% visible and only 7% UV. Space is a nutritional wasteland with respect to water and organic compounds, although comets may provide an oasis when passing a warming star.

Terrestrial organisms most likely to survive these conditions are microbes, with comets or meteorites as conveyance. Microgravity is not lethal; cold tolerance and anhydrobiosis are survivable. Until we understand transit times, we cannot address adequately the nutritional needs of organisms in transit, but we hypothesize that with the exceedingly low metabolic rates that would result from the extremes in cold and desiccation, nutritional needs would not exist. Thus, we are left with two potential ‘show-stoppers’: radiation and the space vacuum. Heavy ions are mutagenic or lethal to microbes. Most damage to microbes exposed to space is due to UV radiation, especially during the short term, but heavy ionizing radiation has a greater probability of being lethal.

Remarkably, some terrestrial organisms can survive this highly extreme environment. This has been proven through flight experiments led by the European Space Agency with American participation (Fig. 6). Microbes tested in the space environment and then returned to Earth include *B. subtilis* spores, bacteriophage T-1, tobacco mosaic virus and most recently osmophilic microbes. *B. subtilis* spores will survive for years in space if either in a bilayer (or multilayer) or mixed with glucose to protect them against high solar UV-radiation flux, but if they are exposed in a monolayer they are killed within minutes. For comparison, viruses lose viability by weeks. Although the data are controversial, *D. radiodurans* did not survive 7 months in space and the DNA had extensive breakage. Halophiles can survive for two weeks in space and probably much longer (R.L.M., M. R. Klovstad, P. Rettrberg, & G. Horneck, unpublished results). The halophiles are the first example of a vegetative cell surviving exposure to the space environment.

**Economic potential of extremophiles**

Extremophiles have provided data that are basic to molecular biology, including information on protein folding. Evolutionary biology has benefited on two fronts. First, in the race to uncover the most extreme of extremophiles, whole new taxa have been discovered, increasing phylogenetic enlightenment. Second, the ability to survive in some extreme environments has evolved multiple times, leading to a new understanding of chance versus necessity in evolutionary pathways, especially at the molecular level. For example, the ice-binding antifreeze proteins are evolutionarily convergent, with that of the Antarctic notothenioid fish evolving from a pancreatic trypsinogen-like protease.

Extremophiles have endeared themselves to multibillion-dollar industries, including agricultural, chemical synthesis, laundry
detergents and pharmaceuticals. The European Commission has supported research, training and the commercialization of technology in this area since 1982. From 1996–1999 it funded the Extremozymes project (see http://www.tutech.de/ecf/ecf_3.html), which is now in a phase of industry-sponsored technology transfer to European companies. G. Antranikian, personal communication. Enzymes are sought to support research, training and the commercialization of novel compounds in industry-sponsored technology transfer to European companies (http://www.tutech.de/ecf/ecf1_3.htm), which is now in a phase of development.

Enzymes from extremophiles – ‘extremozymes’ – have potential in multiple areas, either by using the enzymes themselves, or by using them as sources of ideas to modify mesophile-derived enzymes. In most cases the reaction medium is aqueous, although in some cases the reaction medium is nonaqueous or by using them as sources of enzymes for synthesizing novel compounds. The fastidious growth conditions for extremophiles mean that it is often economically advantageous to express the gene in a more tractable host organism such as E. coli.

The canonical example of extremophile-derived enzymes in biotechnology is the source of Taq polymerase, the enzyme at the crux of the widely used polymerase chain reaction (PCR). Taq polymerase was isolated from the thermophilic bacterium *Thermus aquaticus*, an organism discovered in 1969 in Yellowstone National Park, Wyoming (ref. 121, Fig. 7). DNA polymerases from other thermophiles have been marketed by Promega Corporation as a product for high-fidelity PCR, with each having its own advantages. Other extremophiles have industrial applications. For example, some Antarctic bacteria produce polysaturated fatty acids, an essential dietary ingredient for many aquaculture species (for example, Atlantic salmon). The bacteria are used to enrich rotifers, a food organism for larval fish. Antarctic bacteria have potential in bioremediation of waters following oil spills, which is a concern in cold waters. *D. salina* is widely used for the commercial production of β-carotenes, which it produces in response to solar radiation, and glycerol, which it produces to counterbalance external osmotic pressure.

Human health may benefit from extremophiles indirectly through biotechnology and bioremediation (Table 3). Direct use includes marketing of dried *Dunaliella* as a nutritional supplement, primarily as an antioxidant. Antifreeze proteins show potential as cryoprotectants of frozen organs.

**What next?**

Extremophile research is entering an exciting phase. The commercial potential has been recognized, but is far from being realized. Our ignorance of microbial diversity coupled with improvements in technology and communication will enhance long-term biological studies in space, improving our understanding of the scope of that formerly inaccessible environment. Colonization and terraforming of Mars will require a supporting biota, and where better to start than with extremophiles? And, when life severs its links to planet Earth it will enter new niches ripe for extremophiles, perhaps joining indigenous extraterrestrial extremophiles.


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