Yesterday: Great moments in evolutionary history



formation of the Earth

### Today: Life in Extreme Environments



What is

# Astrobiology?



Overview: Rothschild, L.J. (2001) "Astrobiology". McGraw Hill Encyclopedia of Science & Technology, 2002. pp. 21-24.

### Introduction

What is an extremophile? Why study them? **Environmental extremes** • How do they do it? • Examples of extreme ecosystems • Space: new categories of extreme environments



plus

- - -

"philos", love

"philos", love

equals

plus

"philos", love

equals

Extremophile

plus

"philos", love

equals

Extremophile

#### plus

"philos", love

equals

Extremophile

Coined by Bob MacElroy, NASA Ames Research Center. Macelroy (1974) Some comments on the evolution of extremophiles. Biosystems 6:74-75. Background: At the time, MacElroy was the biochemist in the Biological Adaptation Branch at NASA Ames. He was interested in life in the atmosphere of Jupiter and Mars, and thus was looking primarily at thermophiles. The ones he collected in Yellowstone turned out to be bacilli. He wondered could tRNA survive at 92-98°C when it should melt long before that temperature? He said it was extremely common to combine Greek and Latin words in fact, the Greeks and Romans did it.

### **Philosophical issues**

- Physical, chemical, biological(?) extremes?
- What is "extreme"? Simply in the eye of the beholder? Evolutionary definition? Objective definition?
- Must extremophiles love extreme conditions?
- Must extremophile apply to all life stages? At all times? Deinococcus and frogs.

because life is based on organic carbon in aqueous solution



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### Why carbon?

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### Why carbon?

• It is the fourth most common element in the universe.

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### Why carbon?

- It is the fourth most common element in the universe.
- It is capable of forming compounds, from CH<sub>4</sub> to DNA.
- Atomic carbon and simple compounds with up to 13 atoms have been either detected in interstellar space by spectrometry or produced in laboratory simulations. These include amino acids and nucleotide bases.

# Why study extremophiles?

- Food preservation.
- Basic research; model organisms for basic research
- Biological warfare.
- Biotech potential.
- Future use in space.
- Biodiversity on Earth. Archaea. Origin of life?
- Mechanisms of survival: divergence or convergence? Apparent convergence by LGT?
- Limits for life in the universe.

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### Our only field site so far







### **Extremophiles & Life Elsewhere**

- Richard Proctor. 1870. Other Worlds Than Ours. Possibly first to connect study of life in extreme environments and life on other planets.
- "If we range over the earth, from the arctic regions to the torrid zone, we find that none of the peculiarities which mark the several regions of our globe suffice to banish life from its surface." (ch. 1)





# extremophiles

### Taxonomic Distribution of Extremophiles

...but these are just a

few examples







### Environmental extremes

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#### 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

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#### 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

#### Desiccation

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#### Salinity

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#### **Chemical extremes**

### Environmental extremes



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#### Desiccation

#### **Chemical extremes**

### Environmental extremes

#### Radiation

#### Pressure

#### Temperature

#### Salinity

#### 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

#### Desiccation

#### **Chemical extremes**

### Environmental extremes
Radiation Oxygen



### Temperature

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# Environmental extremes

Radiation Oxygen



### Temperature

### Salinity

### 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

### Desiccation

### **Chemical extremes**

**Electricity** 

# Environmental extremes

### Categories of extremophiles

| Environment | Туре   | Definition   | Examples   |
|-------------|--|--|--|
| Temperature | hyperthermophile<br>thermophile<br>mesophile<br>psychrophile | growth >80°C<br>growth 60-80°C<br>growth 15-60°C<br>growth <15°C | Pyrolobus fumarii -113°; strain 121<br>Synechococcus lividis<br>humans<br>Psychrobacter, insects |
| Radiation   |  |  | D. radiodurans   |
| Pressure    | barophile<br>piezophile                                      | Weight loving<br>Pressure loving                                 | Shewanella viable at 1600 MPa  |
| Desiccation | xerophile  | Cryptobiotic; anhydrobiotic                                      | Lyngbya, tun state of tardigrades  |
| Salinity    | halophile  | Salt loving (2-5 M NaCl)   | Haloarcula, Dunaliella<br>Spirulina, Bacillus firmus   |
| рН          | alkaliophile<br>acidophile                                   | pH >9<br>Low pH loving   | Spirulina, Bacillus firmus OF4 (10.5); 12.8?<br>Cyanidium, Ferroplasma                           |
| Oxygen      | anaerobe<br>aerophile  | Cannot tolerate O <sub>2</sub>                                   | Methanococcus jannaschii<br>Homo sapiens   |
| Chemical    |  | high CO <sub>2</sub> , arsenic, mercury                          | Cyanidium caldarium (pure CO <sub>2</sub> )  |
| Vacuum      |  |  | tardigrades  |
| Electricity |  |  |  |

# Food preservation.

Food preservation is a way to go beyond the limits for life by chemically or physically treating food so that nothing grows in it.

**References:** 



Pickled, Potted and Canned

Jay, James M. 2000. Modern Food Microbiology (6th Edition) . Springer - Verlag

Hugo W.B. 1995. A Brief History of Heat, Chemical and Radiation Preservation and Disinfection. International Biodeterioration and Biodegradation, 36: 197-217(21)

### Categories of food preservation

| Environment          | Method   | Effect on microbes   |
|----------------------|--|--|
| Temperature          | Pasteurization and appertization; cooking<br>Refrigeration<br>Freezing | Delivery of sufficient heat to inactivate targeted microbes to the<br>desired extent<br>Retard growth<br>Retard growth and reduce water activity                           |
| Radiation            | Food irradiation   | Treatment with ionizing radiation. Destroy biomolecules  |
| Pressure             | Pascalization  | Pressure inactivation of vegetative bacteria, yeasts and molds   |
| Desiccation          | Drying, curing   | Reduction in water activity sufficient to delay or prevent growth  |
| Salinity             | Salting, brining   | High osmotic pressure, low water activity  |
| Osmotic              | sugar preservation   | High osmotic pressure, low water activity  |
| рН                   | Addition of acids  | Reduction of pH value and sometimes additional inhibition by the particular acid   |
| Oxygen               | Lactic fermentation oxygen-free or modified atmosphere                 | Delay or stop growth of anaerobes  |
| Chemical<br>extremes | Ethanol, preservatives - nitrite<br>CO2-enriched atmosphere smoke      | Toxic inhibition; can be combined with sugar preservation<br>Inhibition of specific groups of microbes Inhibition of some<br>microbes by carbon dioxide. 'Toxic inhibition |
| Vacuum               | Vacuum   | Delay or stop growth of anaerobes  |



# Temperature

### History of high temperature extremophile research Tom Brock, Karl Stetter

- Brock's first visit to YNP was July 1964. He was surprised by organisms in run-off channels. Stopped again to sample later in the summer. Started to do work in YNP to get field experience before field trip to Iceland. Studies in photosynthesis, chlorophyll, RNA, DNA and protein.
- 1965 tried to culture at high temperature using spring water as culture medium and springs as incubators (didn't work.) Noticed pink, gelatinous mat at Pool A (Octopus Spring.) High temp at the time was considered 60°C.
- Fall 1965 awarded NSF funding, and serious YNP work started 1966. Hudson Freeze cultured YT-1, later known Thermus aquaticus.
- Near end of summer of 1967, starting using immersion slide technique. String in source of Octopus - first evidence for life growing in source. Later than summer showed life in boiling water.
- Stetter's original interest was looking at polymerases in Woese's newly-proposed Archaea.



### History of Deep Sea Extremophile Research - Alvin

In 1977, Alvin traveled through the Panama Canal for the first time. Geology work in the Galapagos Rift was completed during February and March. Discovery of an abundance of exotic animal life on and in the immediate proximity of warm water vents prompted theories about the generation of life. Since no light penetrates through the deep waters, scientists concluded that the animal chemistry here is based on chemosynthesis, not photosynthesis.

Alvin has now found > 24 hydrothermal sites in the Atlantic and Pacific Oceans. It has allowed researchers to find and record about 300 new species of animals, including bacteria, foot-long clams and mussels, tiny shrimp, arthropods, and red-tipped tube worms that can grow up to 10 ft long in some vents.





# Temperature: what difference does it

Solubility of gasses goes down as temperature goes up (impetus for colonization of the land?).

Organisms have upper temperature limits. Chlorophyll, proteins and nucleic acids denature at high temperatures.

Enzymes have optimal temperatures for activity; slow down at low temperature

Low temperature water freezes. Breaks membranes, increases solute concentration, etc.



# **Temperature limits for life\***

*Methanopyrus kandleri\** Strain 121



\*High growth at 20 MPa. Takai et al., 2008. PNAS. \*\*Note many organisms, including seeds and spores, can survive at much lower and higher temperatures.

mesophiles

50

# **Octopus Spring**

# Chloroflexus, 650 Source, > 95°C Thermocrinis

Octopus Spring, Yellowstone National Park, 4 July 1999

# Sol de la Mañana



# Lassen - Snow algae (watermelon snow)







#### • King's Creek, July 2005

### pH limits for life pH is as -log10[H<sup>+</sup>]. At pH 0, [H<sup>+</sup>] = 1 M







# Salinity

- Halophiles: 2-5 M salt
- Include Archaea and a eukaryote.
- Dunaliella salina is used in biotech industry. Produces glycerol and β-carotene.
- Bacterial halophiles were flown in space.







# Laguna Colorada



- Samples were collected in Nalgene containers
- Quite possibly have new species



## Desiccation

- Can be correlated with salinity tolerance.
- Cell growth at normal temperatures usually requires water potential, aw (defined as pH20 [liquid solution] / pH20 [pure liquid water], where p is the vapor pressure of the respective liquid) of >0.9 for most bacteria and >0.86 for most fungi.
- Lowest value known for growth of a bacterium at normal temperatures is aw = 0.76 for Halobacterium.
- Possibly a few organisms, e.g. lichens in the Negev Desert, can survive on water vapor rather than liquid water.
- Don't repair cell damage during desiccation, so must be good at repair upon rehydration.



#### Evaporite, Baja California Sur







# Radiation

- Some forms of radiation have been a constant for organisms over geological time, whereas others vary seasonally and diurnally. Exposure may depend on ecology.
- Man-made radiation can result in novel exposures or exposure levels.
- Some radiation is blocked by the Earth's atmosphere, and thus is newly relevant with respect to interplanetary travel or to an potential extraterrestrial biota.

### The Solar Spectrum



### The Solar Spectrum



### The Solar Spectrum



#### On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life. Charles Darwin,

M.A., Fellow of the Royal, Geological, Linnæan, etc. societies; Author of "Journal of researches during H. M. S. Beagle's Voyage round the world. London: John Murray, Albemarle Street, 1859"

Preface

Introduction

Chapter 1 - Variation Under Domestication Chapter 2 - Variation Under Nature Chapter 3 - Struggle for Existence Chapter 4 - Natural Selection

RADIATION IS UNUSUAL in that it is both a mutagen and a selective agent.



# UV effects: growth rate of yeast under solar radiation



Parent strain, *S. cerevisiae* obtained from Research Genetics. Yeast grown in 50% YEPD, full solar radiation. Jackie Garget, 22 March 2000. UVB had most effect.

# High oxygen

- Oxygen is the one environmental extreme that we consider "NORMAL"
- Actually this is one of the WORST environmental extremes.
- Conclusion: WE are extremophiles too.

$$O_2 \rightarrow O_2^- \rightarrow H_2O_2 \rightarrow OH_2 \rightarrow H_2O_2$$

# Aerobic metabolism: a critical evolutionary innovation

- Aerobic metabolism is more efficient than anaerobic
- Without it, no oxygenic photosynthesis
- Without it, the origin of large animals is unlikely







# Australia

### Brisbane Arkaroola

Sydney

Adelaide

Wednesday, August 4, 2010

Perth

# Australia



### Brisbane Arkaroola

Sydney

• Adelaide

# Australia





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## Paralana Springs

## Paralana Springs

100

 Major challenges include: desiccation, temperature, ultraviolet radiation, trace gases in the atmosphere & "open air effect"

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- Military involvement.

- Major challenges include: desiccation, temperature, ultraviolet radiation, trace gases in the atmosphere & "open air effect"
- Pasteur early work.
- Military involvement.
- Today preservation & disease spread.



Sife is Sazy

## \*efficient

# Easiest thing is to keep the external environment out

- Low pH organisms (acidophiles) use a proton pump to keep their cytoplasmic pH nearer neutral.
- Low temperature organisms (psychrophiles) use antifreezes to prevent ice crystal formation, and cryoprotectants to allow freezing in an organized manner. (note: this has applications from blood preservation to ice cream production)

#### Ways to deal with UV damage...

#### Avoid damage



living under UVabsorbing material (water, dead cells...) production of UV-absorbing pigments (i.e., scytonemin, MAAs, phycoerythrin)





production of quenchers, antioxidants, enzymes to neutralize radicals

> Repair damage

Geysers, vents Ice, in or beneath **Polar regions**  Subsurface **High altitude High salt** High oxygen • Mine drainage Nuclear reactors Soda Lakes Atmosphere

Examples

extreme

Where we have been

#### Lassen SF Bay

Baja California

Galathea 3

Bolivian altiplano

Kenya

New Zealand A Australia



#### preparing to dive under Lake Hoare

#### Lake Hoare, Dry Valleys **Antarctica**



under Lake Hoare





lift-off microbial mat

mat layers

### **Biota of with geysers**

- Biota depends on pH, temperature (distance from source), chemical composition.
- Ex: When the correct combination of temp (~40°C) and pH (~2.5-3.5) exists, Cyanidium caldarium is found.





## pH limits for life - Kenya



- Lakes of the Rift Valley have some of the highest pH in the world.
- Also usually high salinity because they are endorheic which means the only route for loss of water is evaporation.

Wednesday, August 4, 2010

# Rift Valley, Kenya 1.07 Chigh pHD



## **Roaring Mountain**



...is home to an acidophilic thermophile community.

The Archaeum, Sulfolobus acidocaldarius, produces sulfuric acid, which hastens the erosion of the mountainside.

# New Zealand





# Where is the highest radiation on earth?



#### If it's northern summer, go to the Himalayas.



Earth Probe TOMS Version 8 Local Noon Erythemal UV Irradiance on June 01, 2005







Goddard Space Flight Center

## Mt. Everest Expedition, 2009



#### Mt. Everest Expedition, Spectra



→June 1, 2005

#### But in the austral summer, go to the SA etc.



Earth Probe TOMS Version 8 Local Noon Erythemal UV Irradiance on June 01, 2005

Wednesday, August 4, 2010





Goddard Space Flight Center

120

180

240

Local Noon Erythemal UV Irradiance (mW/m2)

300 360

420>

Goddard Space

Flight Center

Earth Probe TOMS Version 8 Local Noon Erythemal UV Irradiance on June 01, 2005

June 1, 2005

Earth Probe TOMS Version 8 Local Noon Erythemal UV Irradiance on January 01, 2005



#### 0 60 120 180 240 300 360 420> Local Noon Erythemal UV Irradiance (mW/m<sup>2</sup>)

Goddard Space Flight Center

#### January 1, 2005

Earth Probe TOMS Version 8 Local Noon Erythemal UV Irradiance on June 01, 2005



–June 1, 2005 🌆 🚽

0 60 120 180 240 300 360 420> Local Noon Erythemal UV Irradiance (mW/m<sup>2</sup>)

Goddard Space Flight Center

## Getting there - San Pedro to Altiplano



## Altiplano, June 2007



# Sol de la Mañana



# Laguna Colorada, Bolivia

- ~ 4500 m high on the Bolivian Altiplano
- Shallow (avg. ~52 cm)
- Area = 5240 hectares
- T = 4-12 °C
- Fresh water springs, but lake mostly saline (average varies 10-20%)
- Saturated with borax



What if we go up?

Challenges include: desiccation, temperature, ultraviolet radiation, trace gases in the atmosphere & "open air effect"


What if we go up?

Challenges include: desiccation, temperature, ultraviolet radiation, trace gases in the atmosphere & "open air effect"

## **Answer: BioLaunch**



#### lowest 100 km of atmosphere



## Why balloons?

- Go through much of stratospheric ozone layer (time travel)
- At 33 km ~ atmospheric pressure of Mars
- Fast (h), inexpensive, repeatable
- Last minute biological sample loading





## Results

#### BioLaunch B07A



## and last week....

- First sounding rocket launch, with Mavericks Civilian Rocketry Foundation Clotho project, and balloon shots (BioLaunch B10A).
- Product launch with Sony and Intel.
- Permits to nearly 100 km for future flights.
- Future: Brazilian flights?





## What next? Basic research

- Continue exploration of Earth
- Survival and evolution of terrestrial organisms in space
- Search for life elsewhere based on what we find on earth.



## What next? Applied research

Big biotech effort on Earth. Search for extremophile biomolecules to improve industrial processes. Use of extremophiles themselves in bioremediation.

Suggest new field: biotech in space. Life support, terraforming. Biomining for synthetic biology. Medical - human limits



| organisms                       | enzyme  | applications  |
|---------------------------------|---|---|
| Thermophiles                    | DNA polymerases   | DNA amplification   |
|                                 | Lipases, pullulanases and proteases   | Baking and brewing  |
|                                 | Xylanases   | Paper bleaching   |
| Halophiles                      | bacteriorhodopsin   | Optical switches and photocurrent generators                    |
| S.cf                            | lipids  | Liposomes for drug delivery and cosmetics                       |
|                                 | Compatible solutes e.g. Ectoin  | Protein, DNA and cell protectants                               |
|                                 | g-Linoleic acid, b-carotene and cell extracts,e.g. Spirulina and Dunaliella | Health foods, dietary supplements, food colouring and feedstock |
| Psychrophiles                   | Alkaline phosphatase  | Molecular biology   |
|                                 | Proteases, lipases, cellulases and amylases                                 | detergents  |
|                                 | Polyunsaturated fatty acids   | Food additives, dietary supplements                             |
|                                 | Ice nucleating proteins   | Artificial snow, food industry e.g. ice cream                   |
| Algaliphiles and<br>Acadophiles | Proteases, cellulases, lipases and pullulanases                             | detergents  |
|                                 | Elastases, keritinases  | Hide de-hairing   |
|                                 | Cyclodextrins   | Foodstuffs, chemicals and pharmaceuticals                       |
| Acidophiles                     |   | Fine papers, waste treatment and de-gumming                     |
| Sulfur-oxidizing acidophiles    |   | Recovery of metals and de-sulphurication of coal                |
|                                 |   |   |



## Suggestion

- Evolution makes its way to localized adaptive peaks.
- Thus, although we have presented a series of onedimensional environmental ranges, in fact the environment is n-dimensional. Some I've mentioned (high temp + acid), others known (e.g., alkaline + saline).
- Probably this n-dimensional space is not filled.

### Concluding thoughts.

- Extremophiles are intimately connected with the fact that we are based on organic carbon in liquid water.
- The story of extremophile research is many stories that have come together in the field of astrobiology.
  - The envelope for life is far beyond what we could have imagined; thus, the habitats for life have been expanded.
- Some adaptations to life in extreme environments are biochemically simple or convergent, suggesting that it could happen elsewhere.
  - The reasonable man adapts himself to the world; the unreasonable one persists in trying to adapt the world to himself. Therefore, all progress depends on the unreasonable man. George Bernard Shaw



"This is Iowa. Io is a moon of Jupiter."



"This is Iowa. Io is a moon of Jupiter."

# NASA's Mantra: Follow the water



category of extreme

Differences in atmospheric composition Altered gravity

- Space vacuum
- Temperature extremes

nun

- Nutrient sources (e.g., organic carbon, nitrogen)
- Different radiation regime (solar and cosmic)





### Why even talk about Venus?

- Earth's "twin" that went bad: lessons for us?
- Chemically lively surface (tectonics, but not plate tectonics) and chemistry
- Potential for past life on surface (Grinspoon, Lonely Planets: the Natural Philosophy of Alien Life)
- Potential for life in the clouds





## Mariners



 Mariner 4 (launched 1964) gave the first glimpse of Mars at close range, putting to rest myths that Mars may have harbored an advanced civilization.







#### The history of Mars and Earth

The Martian Dynamo was Active Prior to 4 Ga, Stopping Solar Wind from Atmospheric ozone stage



#### Why Europa? Liquid water, charged particle-induced chemistry, volcanic activity(?)



Radius of Europa: 1565 km, a little smaller than our Moon's radius. Thin, disrupted, ice crust. Images collected in 1996 by Galileo.



A mission could be devised that would drill through the ice layer and release a probe into the liquid underneath. This is the "hydrobot" concept. http://www.resa.net/nasa/europa\_life.htm



#### Headline, 14 January 2005: Huygens' has touched down on Titan

Huygens' primary goal was to return information on the atmosphere; data about the surface was a bonus. (Image: ESA)

#### Composite of Titan's varied terrain When the probe landed, it was not with a thud, or a splash, but a 'splat'. It landed in Titanian 'mud'.



- This composite was produced from images returned January 14, 2005, by the European Space Agency's Huygens probe during its successful descent to land on Titan. It shows the boundary between the lighter-colored uplifted terrain, marked with what appear to be drainage channels, and darker lower areas.
- These images were taken from an altitude of about 8 kilometers (about 5 miles) and a resolution of about 20 meters (about 65 feet) per pixel. The images were taken by the Descent Imager/Spectral Radiometer, one of two NASA instruments on the probe.

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## Enceladus

"Cold Faithful" found by Cassini team, and reported Feb. 2006

#### Enceladus "Cold Faithful" found by Cassini team, and reported February 2006!

There is sodium and negatively charged water molecules in plumes supporting idea of water ocean (Coates et al., Icarus)



This false-colour image shows the extent of the active region (Image: Nasa/ JPL/S)

> The heat emission is associated with the tiger stripe features which mark the southern pole. The temperatures are in Kelvin (zero K equals -273C).

80 79 80 81 🤮

Mhat about



# Easy to talk about pre-requisites, but we have yet to create life

## Concluding thought, part 2.



#### A good start in the literature

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- Human adaptation: Frances Ashcroft (2001) Life at the Extremes. The Science of Survival. Flamingo Press, London. 326 pp.


## horizon are we alone in the universe?

The hunt for second Earth Tuesday 4 March at 9pm on BBC TWO 'Told via stunning visuals and a playful narrative'

Written & Directed Gideon Bradshaw Camera Kevin White Sound David Strayer Edited Darren Jonunas & Martin Johnson Graphics Jason White & Rob Chiu Research Tom Ranson Horizon Editor Andrew Cohen