Bi 1 Week 8

Symbiosis / Ecology
Symbiotic Relationships of Plants and their Microbiome

Recall Ann Hirsh’s lecture at the beginning of the term.

**Today’s Topic**

**Biological nitrogen fixation** is the reduction of atmospheric $\text{N}_2$ to ammonia by the oxygen-sensitive enzyme nitrogenase.

$$\text{N}_2 + 8\text{H}^+ + 8\text{e}^- + 16\text{MgATP}^{-} \rightarrow 2\text{NH}_3 + \text{H}_2 + 16\text{MgADP}^{-} + \text{P}_1^{-}$$

Plant genomes lack genes encoding the enzyme nitrogenase, which occurs only in eubacteria and archaea. Also, not all plants associate with nitrogen-fixing bacteria, e.g., Arabidopsis.

Even within the bacteria, certain bacteria (free-livers such as Klebsiella, Azospirillum, Azotobacter), cyanobacteria (Anabaena) as well as a few symbiotic species are known nitrogen-fixers: Rhizobium, which nodulates legumes and the Gram-positive actinomycete (Frankia), which nodulates actinorhizal plants such as alder (Alnus), Ceonothus, and Casuarina. **Rhizobium has been the paradigm for understanding symbiotic nitrogen fixation (SNF) to plants.**
Nitrogen Fixation

Nitrogen is a building block of life.

Do you know how much of our atmosphere is made of nitrogen?
Nitrogen Fixation

*The Atmosphere:* 78 percent $N_2$

*But...* Plants need $NH_3$
Nitrogen Fixation

*The Atmosphere*: 78 percent $\text{N}_2$

*But...* Plants need $\text{NH}_3$

\[
\begin{align*}
\text{:N≡N:} + 3\text{H−H} & \rightarrow 2\text{H−N:} \\
\Delta G^0 &= -45.2 \text{ kJ / mol } \text{NH}_3 \\
E_A &= 420 \text{ kJ / mol (without catalysis)}
\end{align*}
\]

*Luckily...* Life uses proteins (enzymes) to catalyze reactions like this!
Nitrogenase

\[ \text{N}_2 + 8 \text{e}^- + 8 \text{H}^+ + 16 \text{ATP} \rightarrow 16 \text{ADP} + 16 \text{P}_i + 2 \text{NH}_3 + \text{H}_2 \]

\[ E_A \sim 400 \text{ kJ/mol NH}_3 \]
Nitrogenase

\[ \text{N}_2 + 8 \text{e}^- + 8 \text{H}^+ + 16 \text{ATP} \rightarrow 16 \text{ADP} + 16 \text{P}_i + 2 \text{NH}_3 + \text{H}_2 \]

\[ E_A \sim 400 \text{ kJ/mol NH}_3 \]

Stepwise Catalytic Conversion (steps have lower \( E_A \))
Nitrogenase Structure

Back to relating protein structure and function:

- Amino acid sequence encoded by *nif* genes (Primary Structure)
- Alpha Helices (Secondary Structure)
- Protein Molecule (Tertiary Structure)
- Protein Complex (Quaternary Structure)
- B-Sheets (Secondary Structure)
Nitrogenase Structure + Function

Back to relating protein structure and function:

The different metal centers enable essential ET throughout the protein in this process.
Nitrogenase Structure + Function

Back to relating protein structure and function:

Different metal centers enable ET throughout the protein process.

FeMoco cofactor

D. Rees (CCE)  J. Peters (CCE)

J. Peters (CCE)
Nitrogenase in Rhizobia

Rhizobia are soil bacteria that fix nitrogen (actively express nitrogenases) after becoming established inside the root nodules of legumes.
Establishing the Partnership

Legume

Rhizobia
Rhizobia

Legume

Establishing the Partnership

Flavonoids are given off by legume roots when they need more nitrogen fixing partners
Establishing the Partnership

Legume

Rhizobia secrete Nod factors

Rhizobia
Establishing the Partnership

Rhizobia Legume

Bacteria enter the plant through root hairs. A nodule then forms around the infection.
Plant Controls $O_2$ Supply to Bacteria

LEGUME

$O_2$ control

BACTERIA

leghemoglobin

PDB 2DGM
Plant Provides Energy for Bacteria
Bacteria provides Fixed Nitrogen for Plant

LEGUME

O\textsubscript{2} control

Energy

Fixed Nitrogen

BACTERIA
Symbiosis has Checks and Balances

LEGUME

O₂ control

Energy

BACTERIA

Fixed Nitrogen

Signaling Pathways
(Think about Ann Hirsh’s lecture)
$N_2$ Fixing Symbiosis in the Deep Ocean

Caltech Lab Link
http://www.gps.caltech.edu/people/vorphan/research/Nitrogen_Fixation
**FISH**
Fluorescence in-situ Hybridization

**Method:** fluorescent probes that bind to certain RNA or DNA sequences

Used to localize the presence and absence of specific RNA or DNA sequences on chromosomes.

Dekas et al. used two different probes:
- **Red:** ANME-2 (target: Eel932)
- **Green:** Desulfobateraceae (target: DSS658)

**NanoSIMS**
single-cell-resolution nanometer secondary ion mass spectrometry

**Target Surface**
FISH
Fluorescence in-situ Hybridization

**Method:** fluorescent probes that bind to certain RNA or DNA sequences

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Dekas et al. used two different probes:
- **Red:** ANME-2 (target: Eel932)
- **Green:** Desulfovibrio (target: DSS658)
rRNA Trees
Trees not Ladders

Fish  Mouse  Gorilla  Human

Human

↑
Gorilla

↑
Mouse

↑
Fish
Phylogenetic trees are like family trees
Tree Terminology

Leaf

Root

Node

Monophyletic clade

A

B

C

D
Different ways to display trees

Unrooted

Rooted

Rooted

Rooted
Are these trees different?
How to make a tree

DNA → rRNA sequence → Alignment → Tree

PCR, Sequencing → ClustalW → PhyML

Seq 1: A G T G T
Seq 2: A G T A T
Seq 3: C G C T G
Seq 4: C G T C A
## Multiple Sequence Alignment by CLUSTALW

<table>
<thead>
<tr>
<th>CLUSTALW</th>
<th>MAFFT</th>
<th>PRRN</th>
</tr>
</thead>
</table>

### General Setting Parameters:
- **Output Format:**
  - CLUSTAL

- **Pairwise Alignment:**
  - FAST/APPROXIMATE
  - SLOW/ACCURATE

### Enter your sequences (with labels) below (copy & paste):
- **PROTEIN**
- **DNA**

Support Formats:
- FASTA (Pearson)
- NBRF/PIR
- EMBL/Swiss Prot
- GDE
- CLUSTAL
- GCG/MSF

### Or give the file name containing your query

- **Browse...**

- **Execute Multiple Alignment**
- **Reset**

More Detail Parameters...
<table>
<thead>
<tr>
<th>Group</th>
<th>Sequences</th>
<th>Score</th>
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<td>7</td>
<td>21616</td>
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<td>13</td>
<td>12</td>
<td>19504</td>
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Alignment Score 1788942

PHYLIP-Alignment file created [clustalw.phy]

```
clustalw.phy

26  2164
Thermococc    ---------------  ---------------  ---------------  ---------------  ---------------
Halobacter    ---------------  ---------------  ---------------  ---------------  ---------------
Methanosar    ---------------  ---------------  ---------------  ---------------  ---------------
20            ---------------  ---------------  ---------------  ---------------  ---------------
Clostridium   ---------------  ---------------  ---------------  ---------------  ---------------
Bacillus_s    ---------------  ---------------  ---------------  ---------------  ---------------
Bacteroides   ---------------  ---------------  ---------------  ---------------  ---------------
Chlorobium    ---------------  ---------------  ---------------  ---------------  ---------------
Legionella    ---------------  ---------------  ---------------  ---------------  ---------------
Pseudomonas    ---------------  ---------------  ---------------  ---------------  ---------------
Yersinia_p     ---------------  ---------------  ---------------  ---------------  ---------------
Serratia_l    ---------------  ---------------  ---------------  ---------------  ---------------
Vibrio_vul    ---------------  ---------------  ---------------  ---------------  ---------------
Magentospi    ---------------  ---------------  ---------------  ---------------  ---------------
Rhizobium     ---------------  ---------------  ---------------  ---------------  ---------------
Chloroplas    ---------------  ---------------  ---------------  ---------------  ---------------
Synechocys    ---------------  ---------------  ---------------  ---------------  ---------------
Mycobacter    ---------------  ---------------  ---------------  ---------------  ---------------
Streptomyce   ---------------  ---------------  ---------------  ---------------  ---------------
```
## Server load: 33%

A new beta version of PhyML is available for download. Click here for details. All comments are welcomed.

### PhyML online execution

#### Input Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Sequences (PHYLIB format)</td>
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<tr>
<td>Data Type</td>
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<tr>
<td>Sequence file</td>
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<td>Number of data sets</td>
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#### Substitution Model

<table>
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<tbody>
<tr>
<td>Substitution model</td>
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<td>Equilibrium frequencies</td>
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<tr>
<td>Transition / transversion ratio (DNA models)</td>
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<td>Proportion of invariable sites</td>
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<tr>
<td>Number of substitution rate categories</td>
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<tr>
<td>Gamma shape parameter</td>
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</tbody>
</table>

#### Branch Support

<table>
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<th>Value</th>
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<td>Perform bootstrap</td>
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<tr>
<td>Name of your analysis</td>
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<tr>
<td>Your email</td>
<td></td>
</tr>
<tr>
<td>Please confirm your email</td>
<td></td>
</tr>
</tbody>
</table>

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Please cite:

Project 1

No description.

Table:

| Tree | Description | Datasets | Saved views | Inserted | Features |

Upload a new tree to this project

Add a new project...


Upload a tree into the project 'Project 1' on workspace 'bi 1'

Use this form to upload your own phylogenetic tree. It should be in plain text, in one of supported formats (Nexiwick, Nexus or PhyloXML). Please check our supported formats and extra features available.

You can either paste your tree into the box, or upload a file using the file selector below. Don't forget to select the correct tree format.

Paste or type the tree:

Optional information:

- Tree name:
- Tree description:

Advanced options (show)

Upload
Tree uploaded successfully

Tree has 50 nodes in total, of which 26 are leaves.

Tree was added to project 'Project 1' in workspace 'bi 1'.

You can now Display the tree, or return to workspace 'bi 1.'

Other options:

These are also available within the tree list in the project box.

- Define color ranges: you can interactively define color ranges for various internal nodes in your tree
- Automatically assign taxonomy: if this tree uses NCBI species taxonomy IDs, ITOL can automatically assign proper scientific names to both internal and leaf nodes.

Please select the output format below and customize other export options as required. The default font size is likely wrong, click the 'suggest optimum' link to calculate the correct value for the current tree view.

<table>
<thead>
<tr>
<th>Format</th>
<th>Encapsulated Postscript (eps)</th>
<th>select the desired output format</th>
</tr>
</thead>
<tbody>
<tr>
<td>Font size</td>
<td>12 pixels (suggest optimum)</td>
<td>font size for leaf labels</td>
</tr>
<tr>
<td>Display leaf labels</td>
<td>yes □ no □</td>
<td>you can omit leaf labels from the exported trees using this option</td>
</tr>
<tr>
<td>Line width</td>
<td>3 px</td>
<td>line width for tree branches, in pixels</td>
</tr>
<tr>
<td>Tree scale factor</td>
<td>1.0 X</td>
<td>stretch or shrink the tree horizontally</td>
</tr>
<tr>
<td>Branch colors</td>
<td>Ignore □ use □</td>
<td>if branch colors are ignored, they will be black by default</td>
</tr>
<tr>
<td>Leaf labels</td>
<td>□ Show original ID □</td>
<td>if leaf labels were edited (or automatically assigned), use this option to show original tree IDs instead</td>
</tr>
</tbody>
</table>

Export tree