Seven Pines 2011

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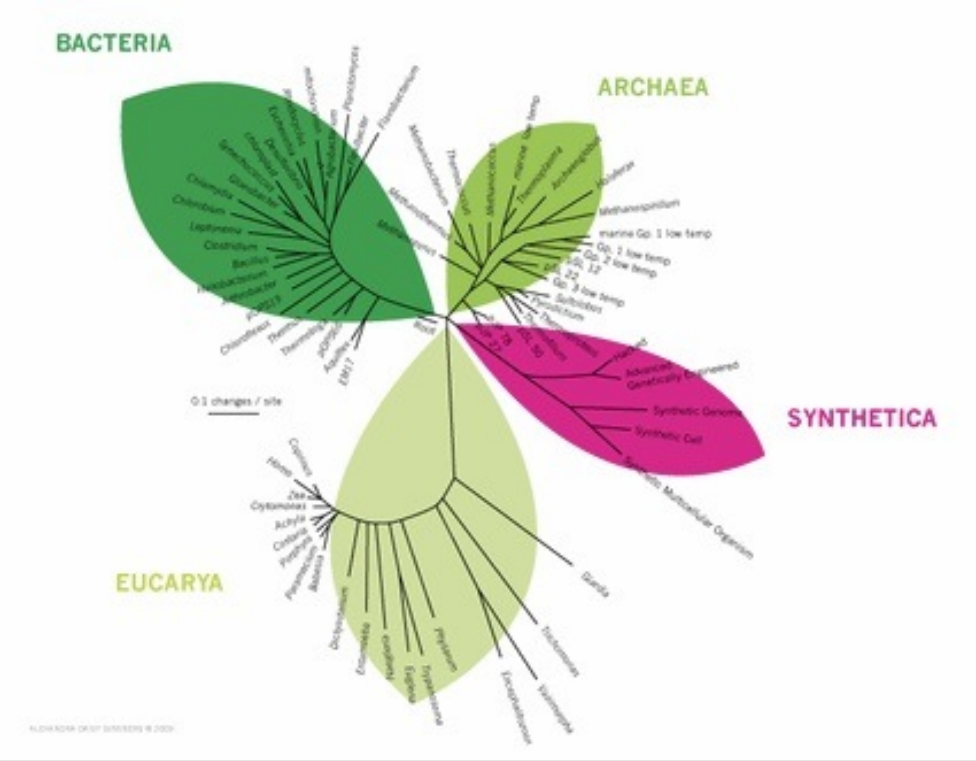
Synthetic Biology

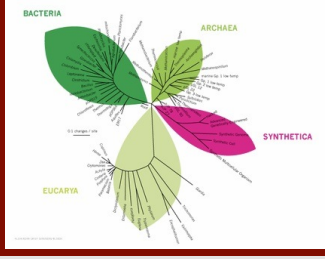
"Combination of science and engineering"

"Design and construction of new biological functions and systems not found in nature"

- Biotechnology
- Hypothesis testing (Minimal Genomes)

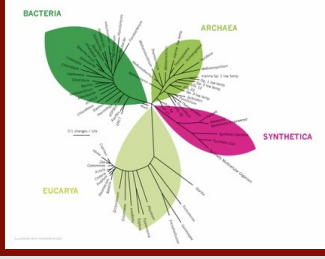


Biotechnology



- Overproduction of useful compounds or design and expression of efficient pathways
- Bio-computation
  - Parallel evaluation
- Directed evolution
  - Pruning of poor solutions

Bio-computation: Parallel evaluation



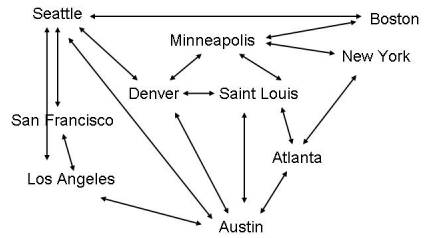
- Brute force computing:
- DNA computing utilizes parallel computing

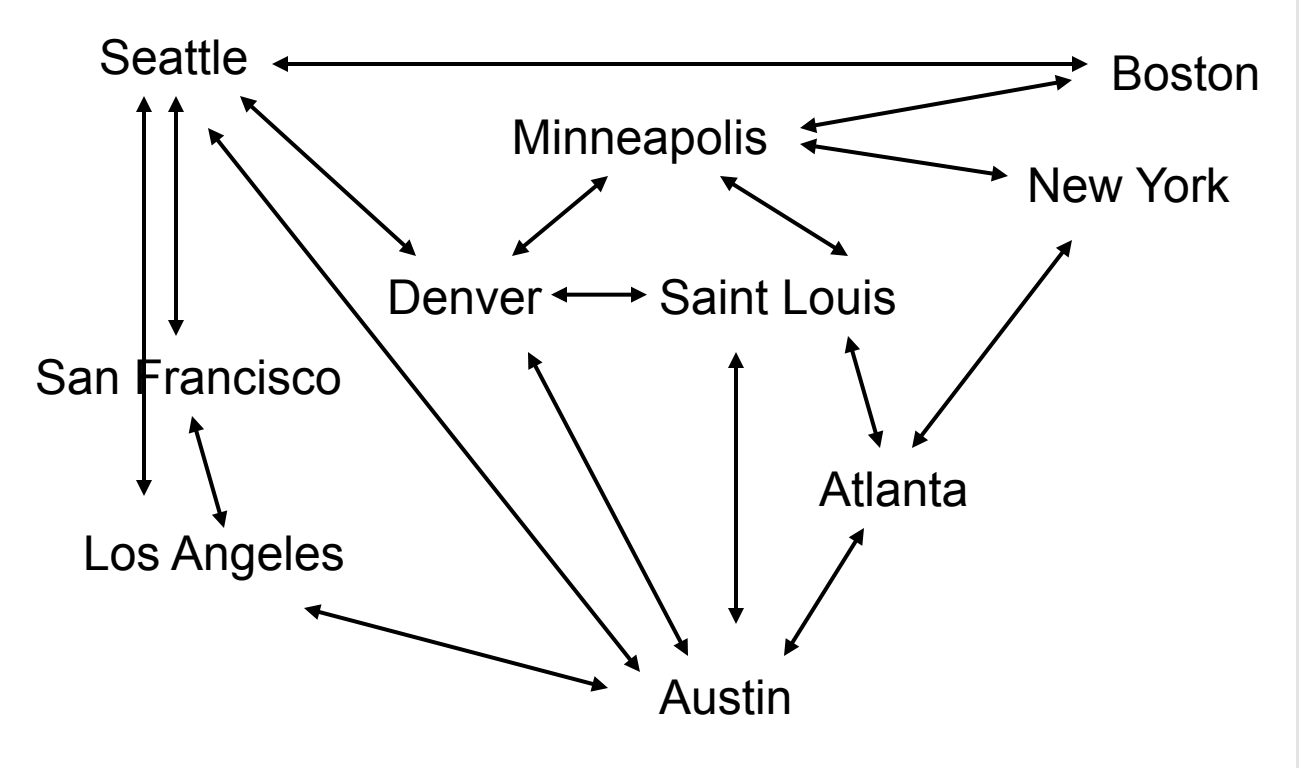
DNA stores 18Mbits/inch

DNA has self matching capability

- Traveling salesman problem
- Encryption problems

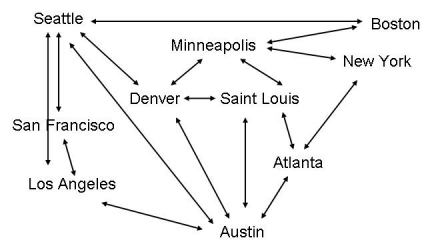
Traveling Salesman Problem





Leonard Adleman: Molecular computation of solutions to combinatorial problems. Science, 266:1021-1024. (Nov. 11). 1994

Algorithm

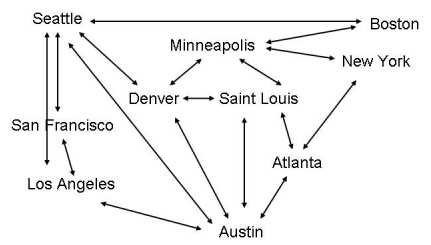


Hamiltonian path problem is NP complete

Algorithmic solution:

- Step 1: Generate random paths through the graph.
- Step 2: Keep only those paths that begin in the origin and end in the final destination
- Step 3: Keep only those paths that enter all of the destinations
- Step 4: Keep the shortest route

DNA Computing



- Palindromes provide a self-matching mechanism:

- Cities can be encoded using a specific DNA sequence

  CCGATCGG = Boston

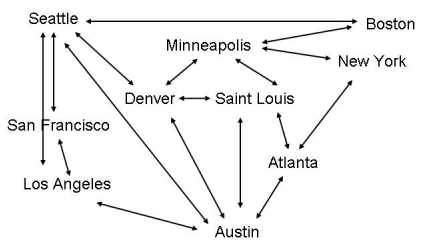
  ACGTACGT = New York

- Distance of itinerary can be coded as spacer (NNNN)

...NNNNNNCCGATCGG TGCATGCANNN...

GGCTAGCCNNNNNNNNNACGTACGT

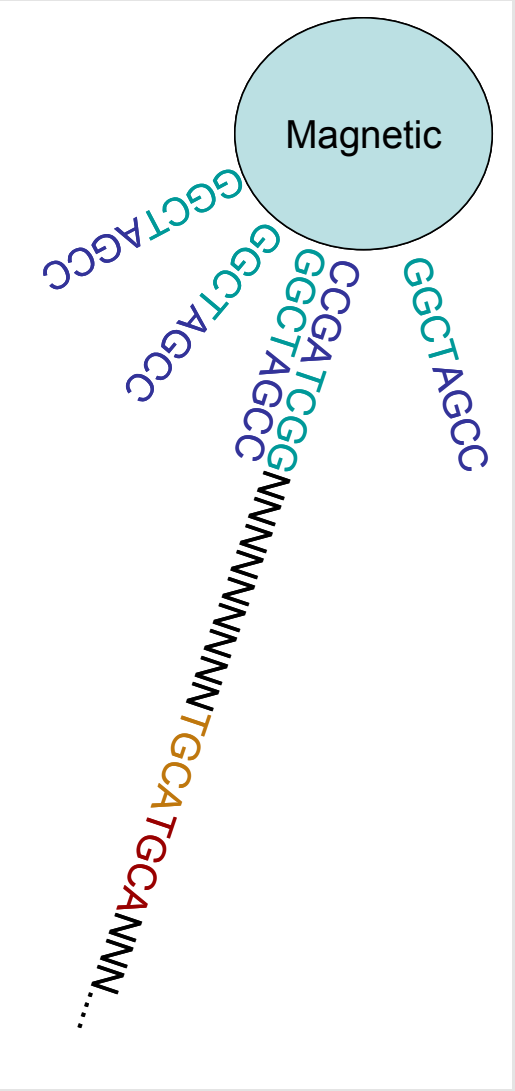
DNA Processing



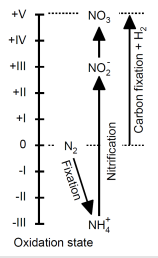
- DNA-ligase produces double strands DNA:

...NNNNNNCCGATCGGNNNNNNNNNTGCATGCA
...NNNNNNGGCTAGCCNNNNNNNNNACGTACGT

- Routes starting with the city of origin and ending in the final destination can be amplified using PCR with matching primers
- Required cities in the itinerary can be evaluated using affinity purification
- Shortest remaining DNA molecule corresponds to shortest route



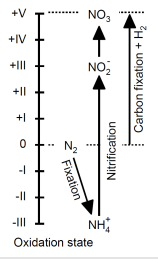
Directed Evolution



- Natural selection for a feature of interest
- Design of the conditions that the feature of interest itself, or a feature linked to it, conveys a benefit

- Excess nitrogen fixation in Anabaena:
  - excess nitrogen fixation is not in the benefit of anabaena
  - How can we align interests

Photosynthetic Nitrogen Fixation



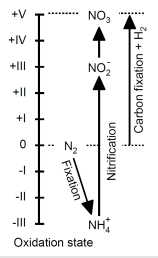
- Why people care:

- Nitrogen is growth limiting in agriculture
- 1-2% of global energy supply is used for nitrogen fixation
- Nitrogenase reduces N_2 to NH_3:

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$

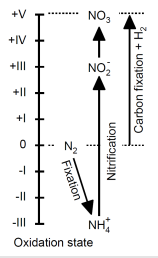
- Photosynthesis can conveniently all required energy and electrons - or not? -

Nitrogenase is oxygen sensitive



- Nitrogen fixation has to occur anoxically
  - Search for oxygen insensitive nitrogen fixation has yielded no results
  - Biotechnological design of a oxygen insensitive variant is implausible
- Nature has dealt with this by:
  - Producing a specialized anoxic companion cell
  - Creating very fast respiration to drive down the cellular oxygen concentration
  - Photosynthesize without producing oxygen.

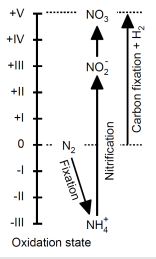
Photolithotrophic Nitrogen Fixation (purple sulfur bacteria)



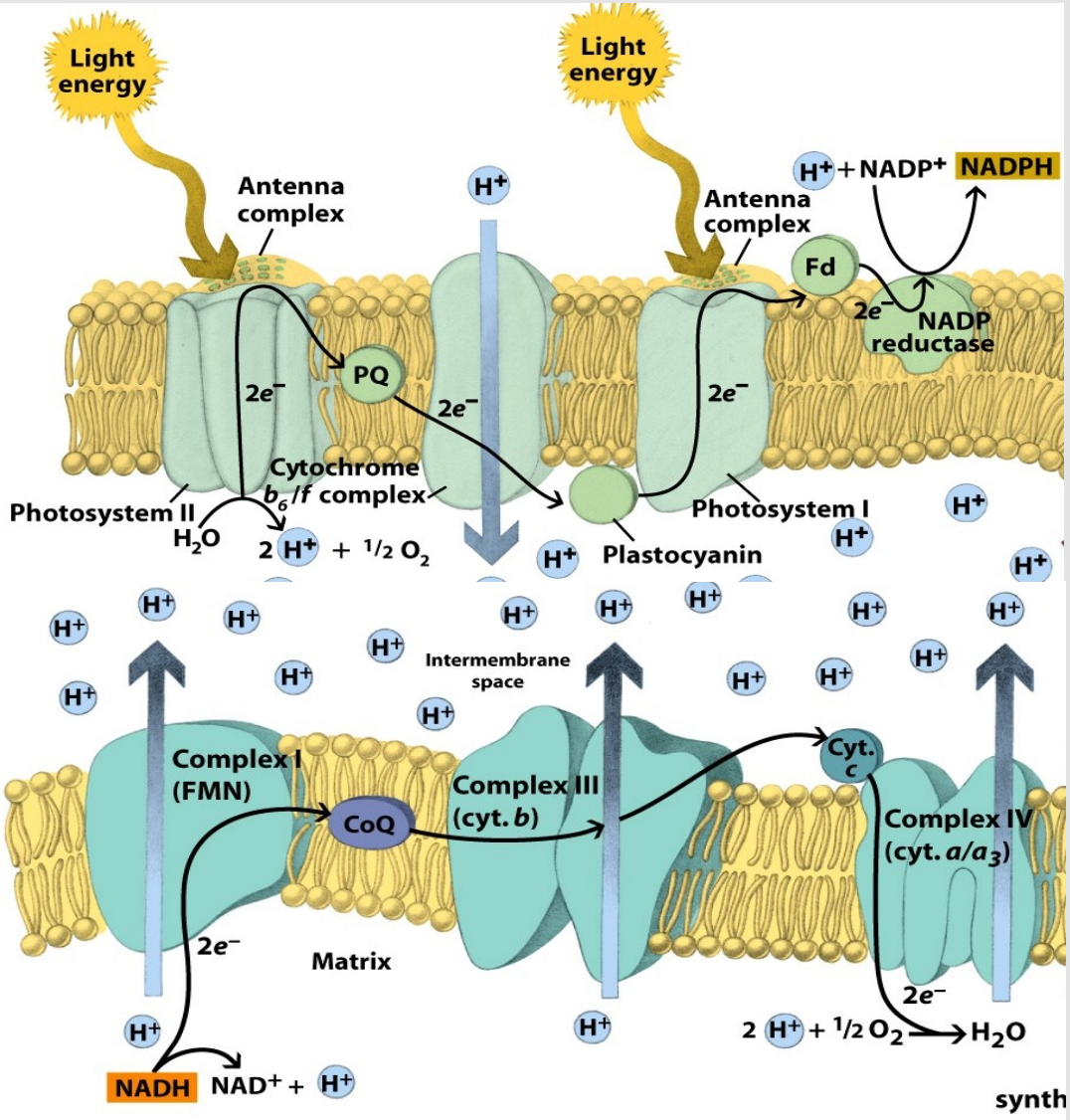
- Energy from photosynthesis
- Electrons from a chemical source (litho = stone)
- No oxygen production, but an external electron source required
- Nitrogen fixation is only beneficial for the organism to the extent that it needs it

Aquatic Microb Ecol 12: 105-113, 1

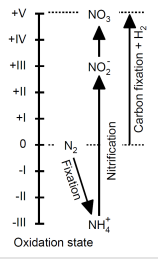
Respiration and Photosynthesis



- Water provides electrons for CO2 reduction
- Oxygen is co-produced
- Respiration oxidizes sugars to produce water
- Components of electron transport chains have common ancestry



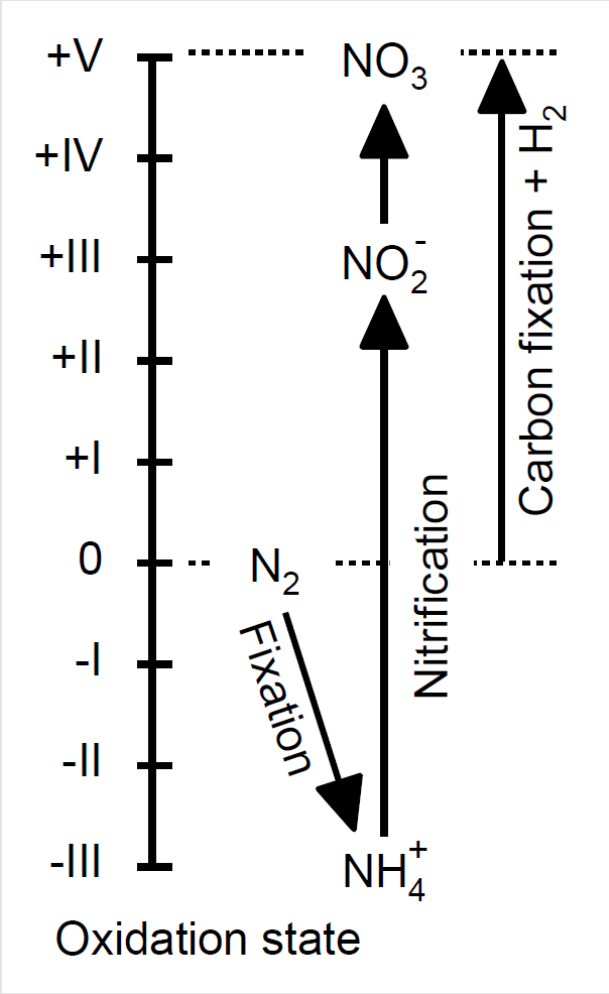
Nitrogen Fixation in Anabaena



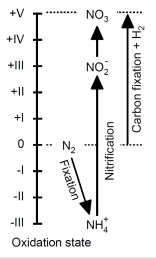
- N_2 is relatively reduced (0)
- NO_3^- is oxidized (5)
- N_2 could provide electrons for biomass production:

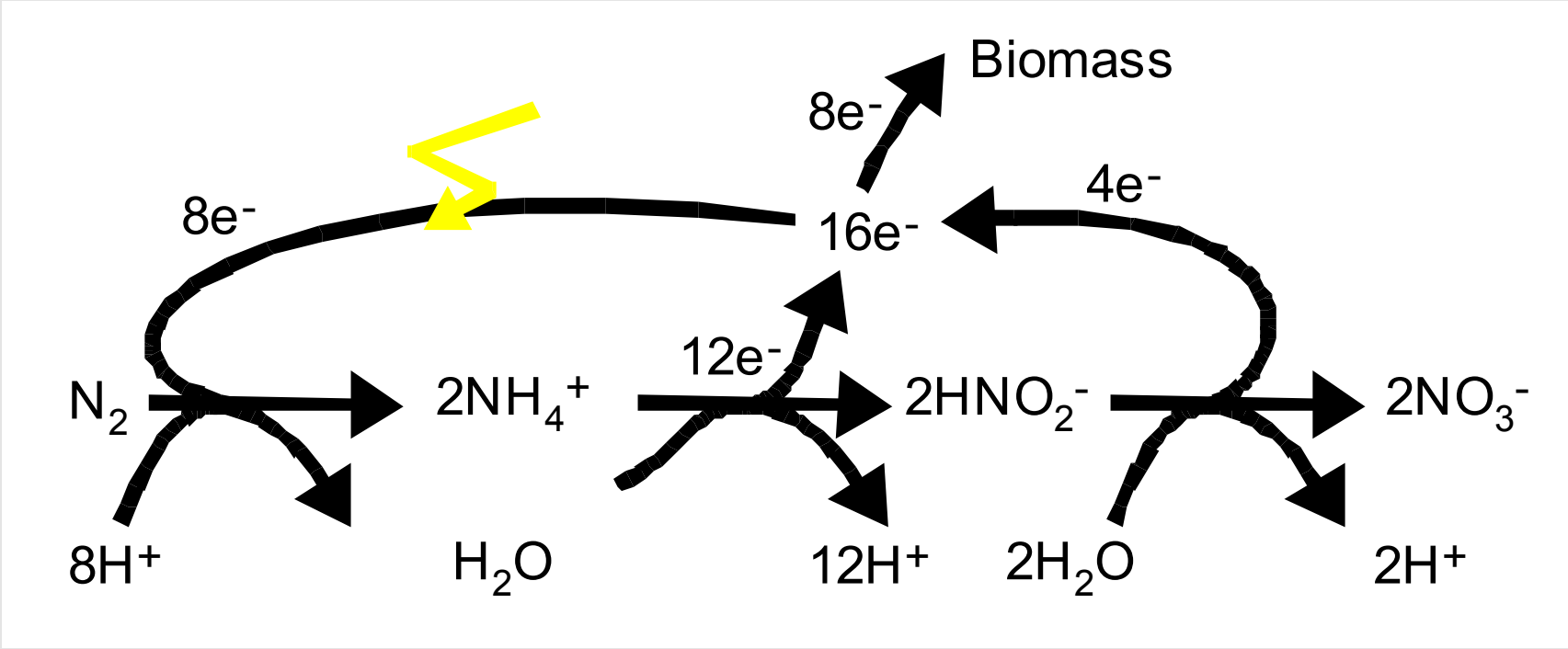
$$3N_2 + 12NADP^+ \rightarrow 6NO_3^- + 12NADPH$$

$$6CO_2 + 12NADPH \rightarrow C_6H_{12}O_6 + 12NADP^+$$

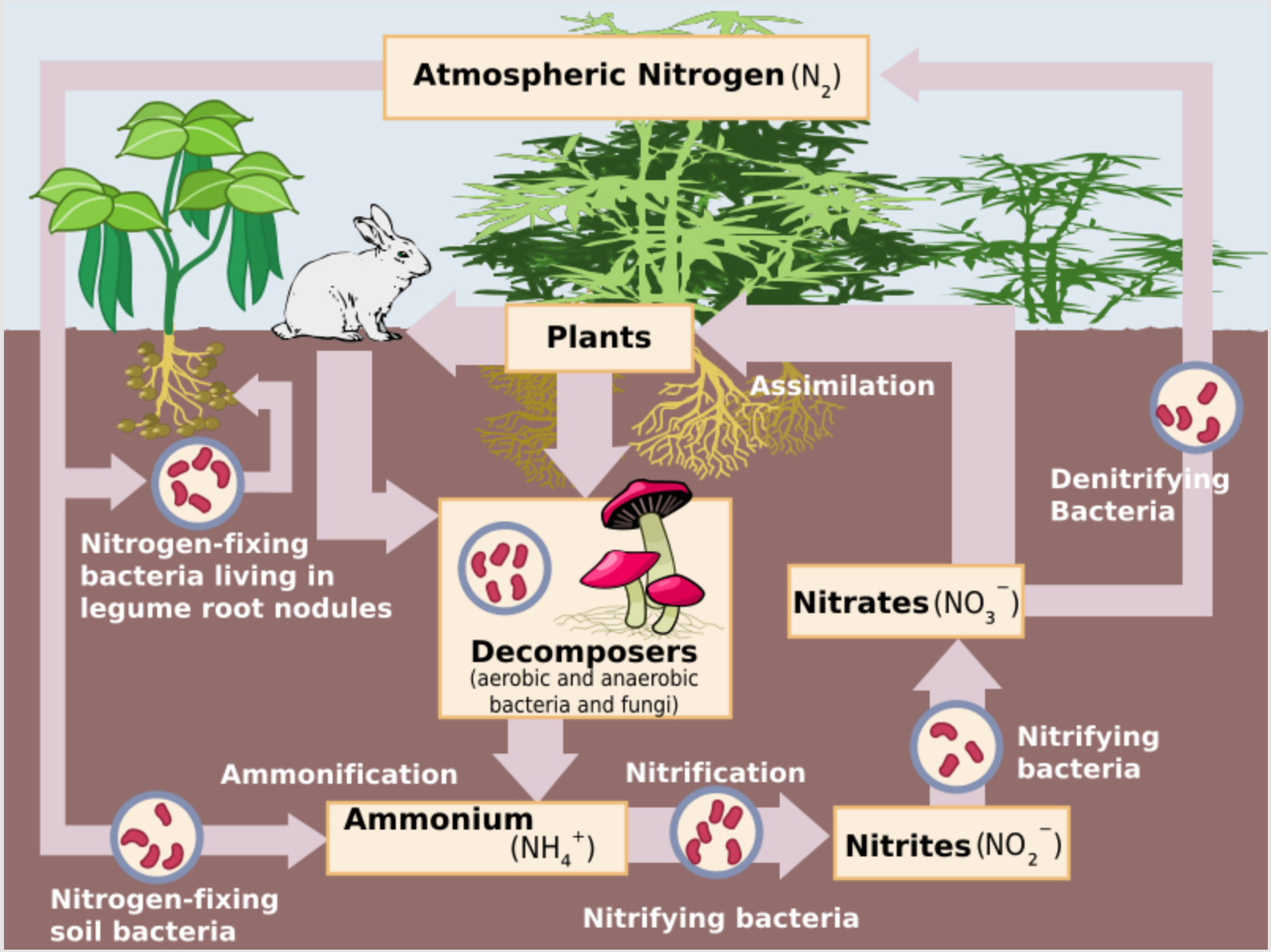


Nitrogen Fixing Electron Cycle

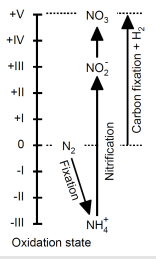


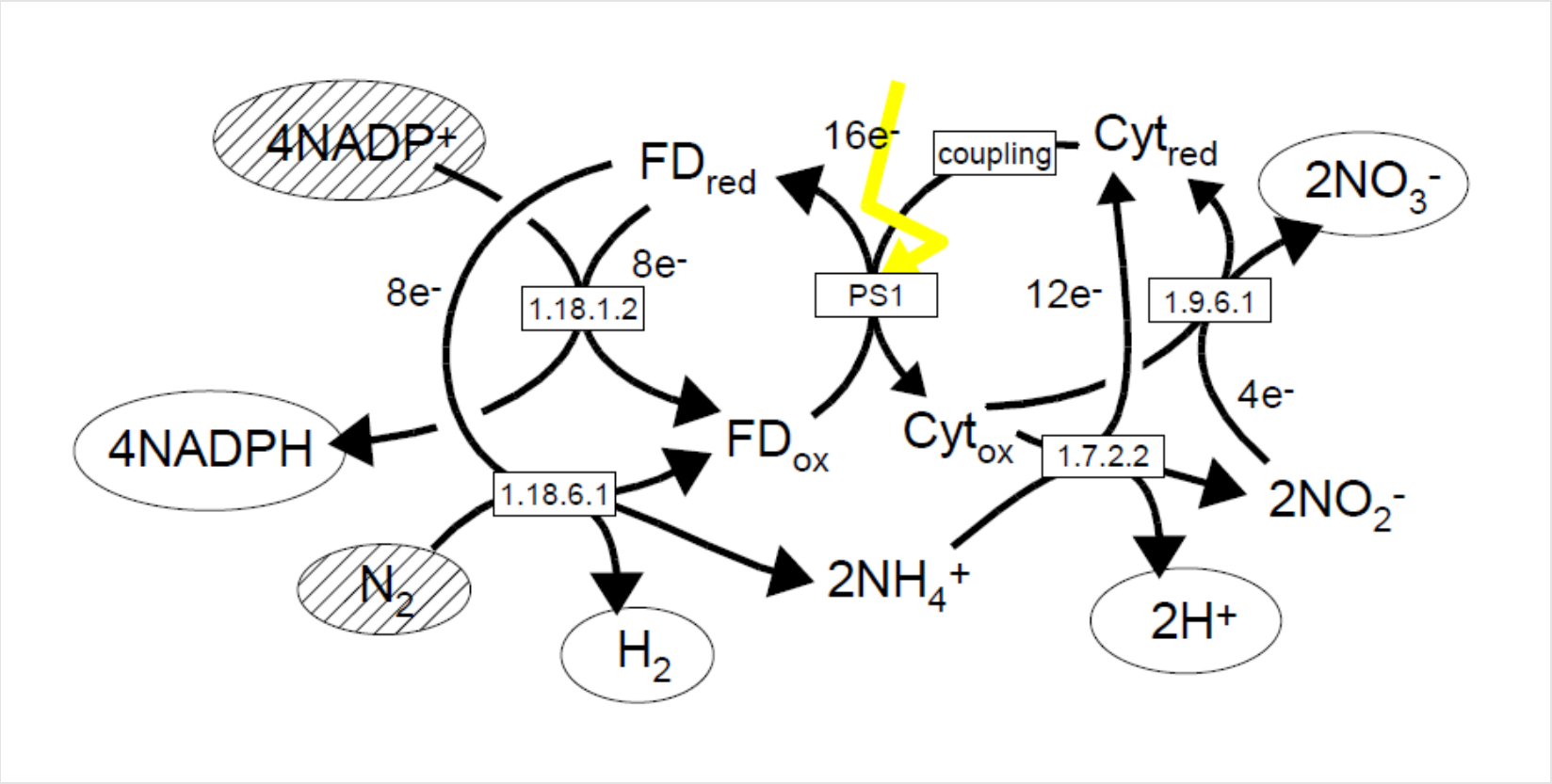


- 8 electrons are recycled to fix N_2
- 8 electrons are available for biomass formation


Atmospheric Nitrogen (N_2)
Plants
Assimilation
Denitrifying Bacteria
Nitrogen-fixing bacteria living in legume root nodules
Decomposers
(aerobic and anaerobic bacteria and fungi)
Nitrates (NO_3^-)
Nitrifying bacteria
Ammonification
Nitrification
Ammonium (NH_4^+)
Nitrites (NO_2^-)
Nitrogen-fixing soil bacteria
Nitrifying bacteria

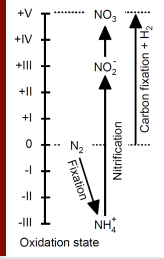
Potential Enzyme System



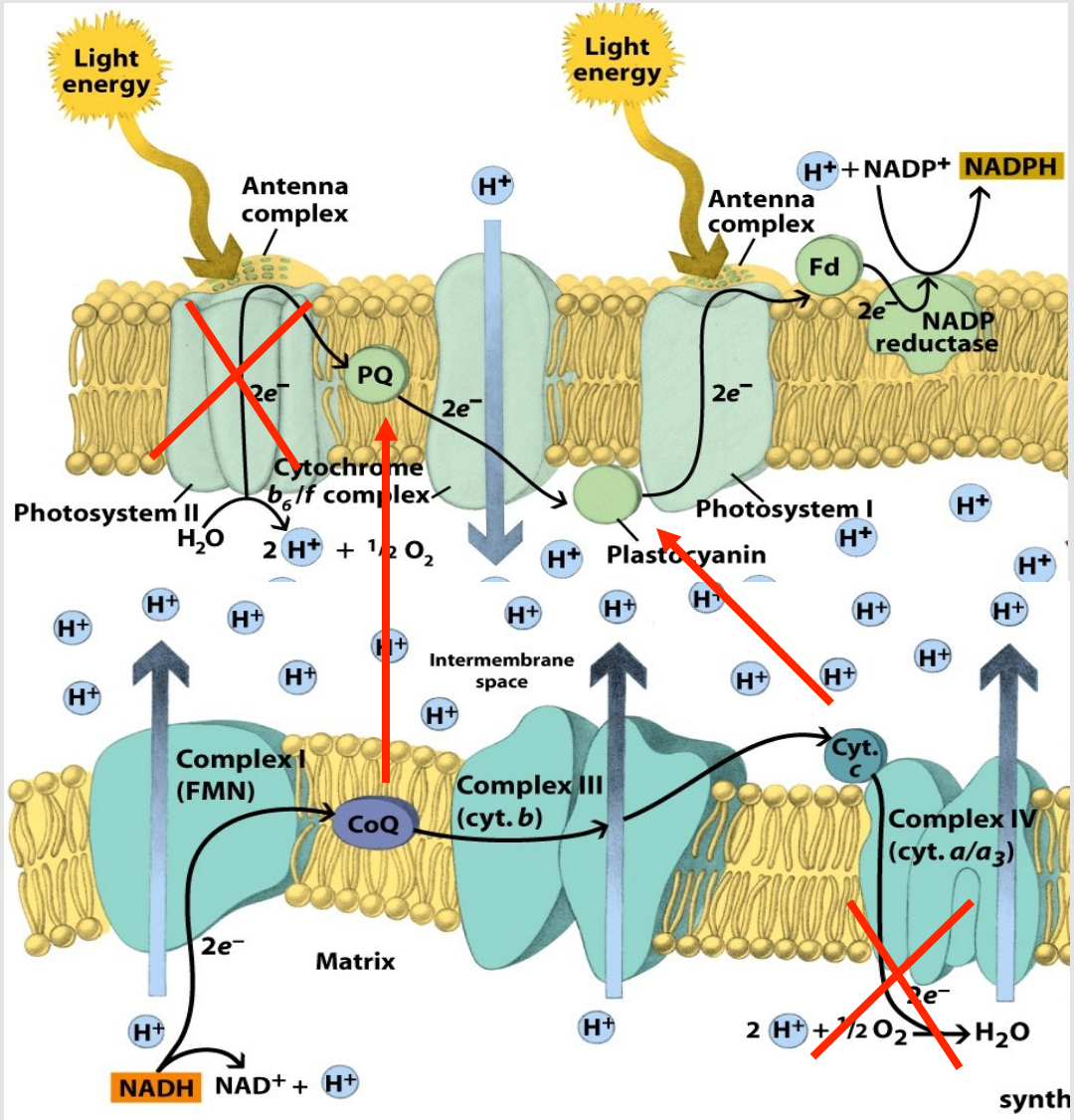


- Reduced CytC needs to donate electrons to the photosynthetic electron transport chain
- Photosystem 1 provides the electronegativity to transfer electrons to ferrodoxin

Potential electron shunts

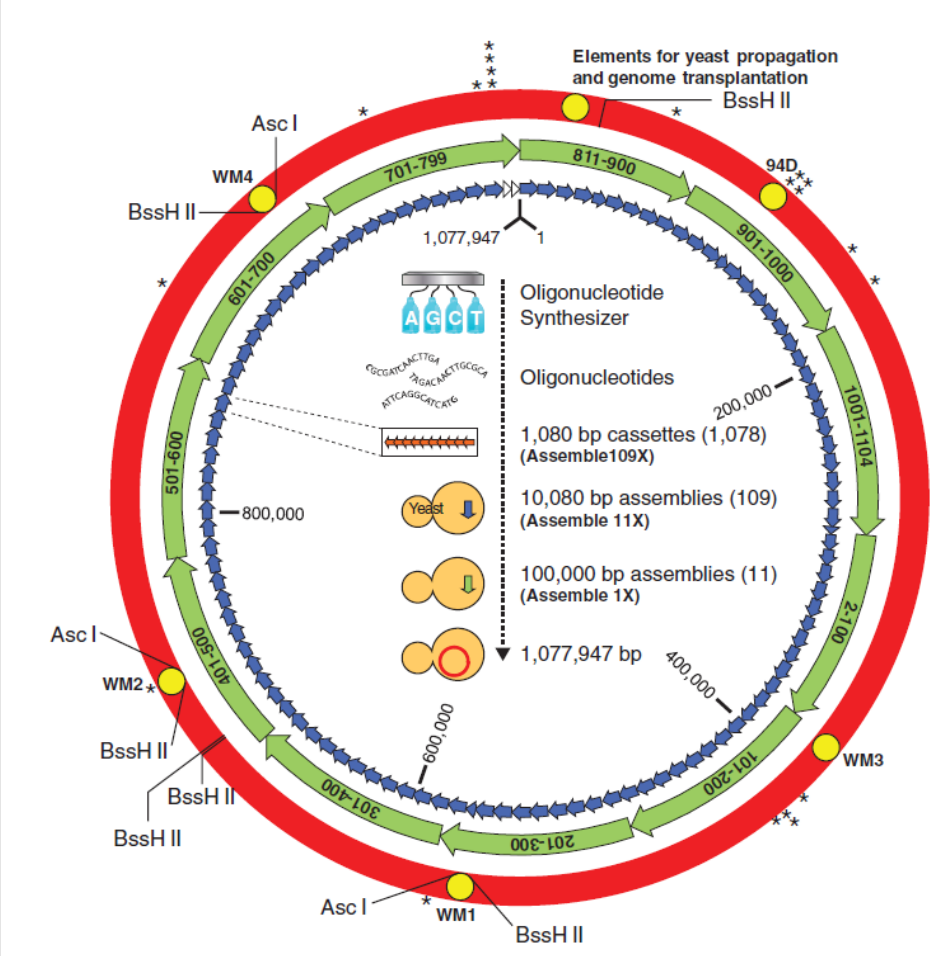


- Q cycle in the respiratory and photosynthetic electron chain are very similar
- Quinones are similar
- CytochromeC and plastocyanin have similar redox potential



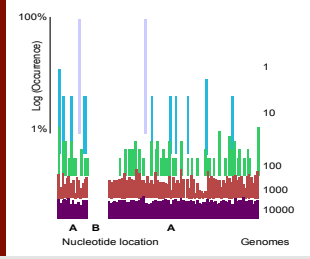
Synthetic genomes (bottom up)

- Demystification of life
- Venter et al. synthesized the genome of *Mycoplasma mycoides* de-novo
- “Water marks” were used to prove the synthetic origin of the DNA
- Synthetic DNA was injected in host cells
- original phenotype was recovered

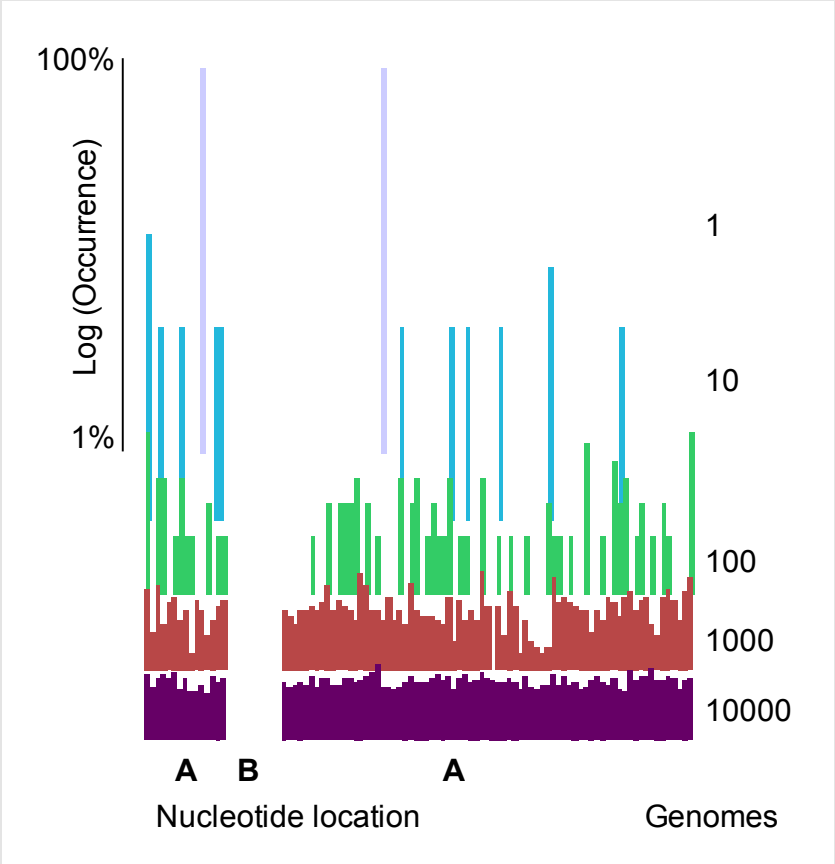


D. G. Gibson *et al.*, *Science* **329**, 52 (2010).

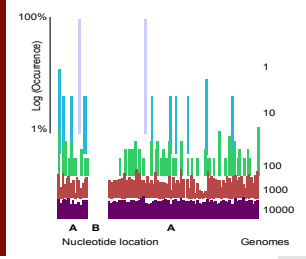
Synthetic genomes (top down)



- Bio-computational approach
- Labeling of unutilized genes with variation
- Deep sequencing of resulting culture
- Gene-wide nonsynonymous mutations indicate random mutations
- “labeled” genes are unlikely to be utilized
- Detection of single nucleotide polymorphisms requires deep sequencing



Fixation rate of mutations is independent of application regime



- Survival fraction (S) is a function of probability of survival per mutation (p) and mutation events per cell per generation (n):

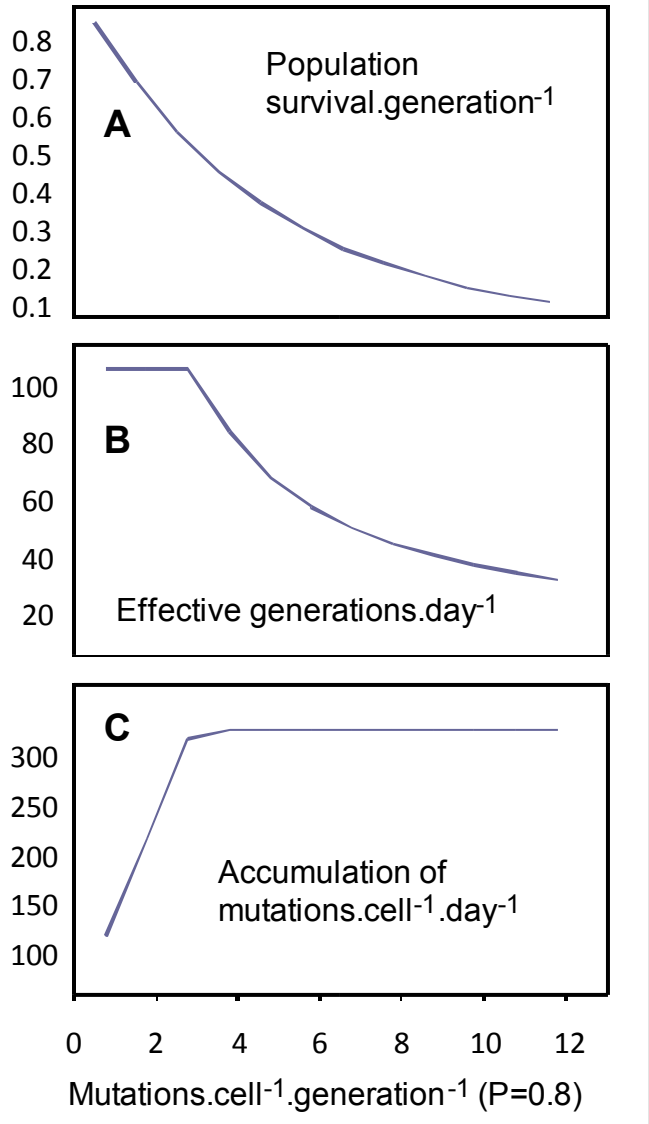
$$S = p^n$$

- survivors accumulate mutations (M) as the product of generations (g) and mutation events (n)

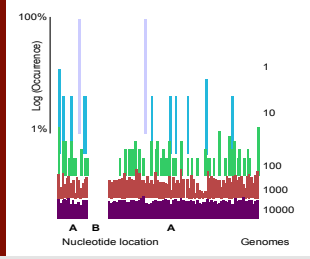
$$M = g \cdot n$$

$$t_r = \log_2\left(p^{-n}\right) = -n \cdot \log_2(p)$$

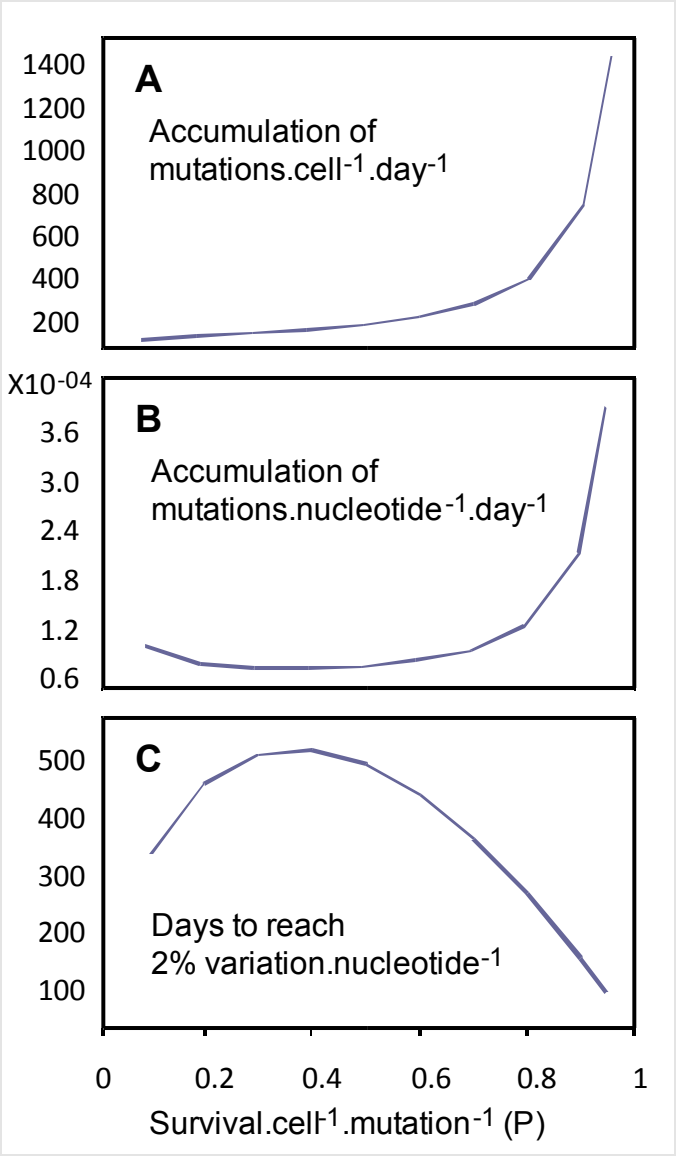
$$M = g \cdot \frac{n}{-n \cdot \log_2(p)} = -g \cdot \log_2(p)^{-1}$$



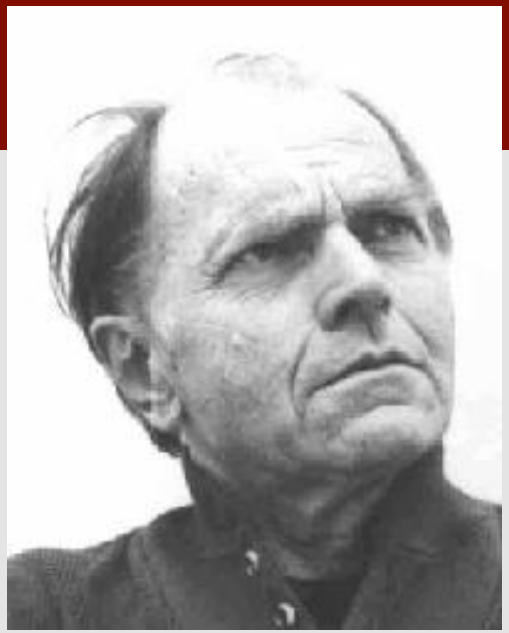
Required Evolution Time



- Accumulation rate of mutations is a function of survival rate
- Accumulation rate per nucleotide has a worst case scenario and two optima
- Required experimental time to reach a given variation is inversely proportional
-



Against Method

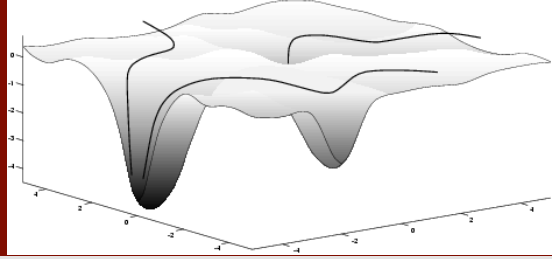


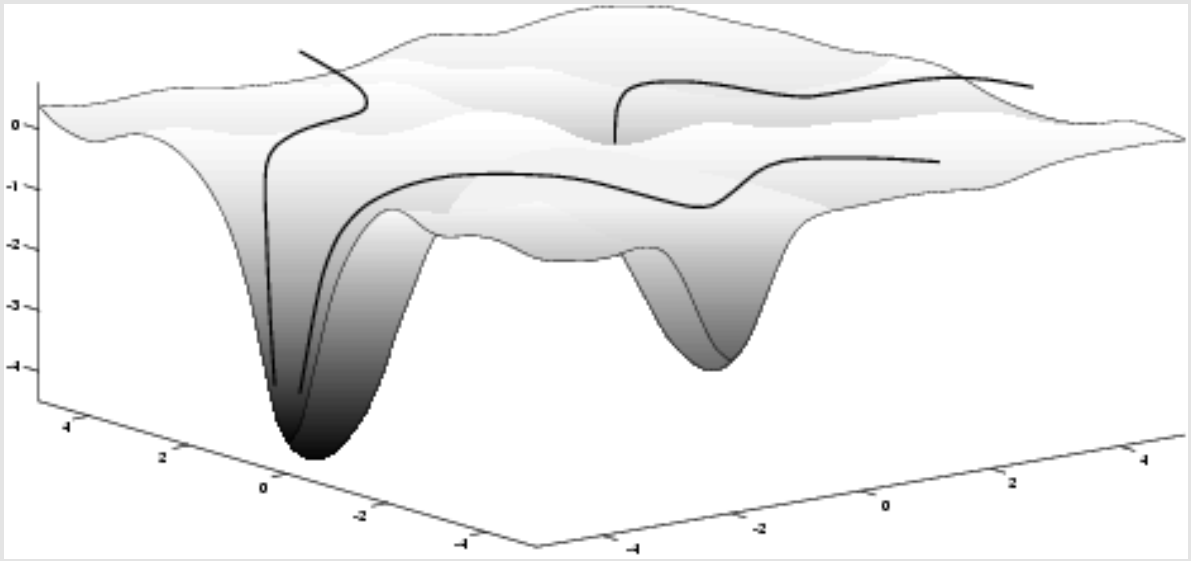
Against Method (1975)

Paul Feyerabend

"The consistency condition which demands that new hypotheses agree with accepted theories is unreasonable because it preserves the older theory, and not the better theory. Hypotheses contradicting well-confirmed theories give us evidence that cannot be obtained in any other way. Proliferation of theories is beneficial for science, while uniformity impairs its critical power. Uniformity also endangers the free development of the individual."

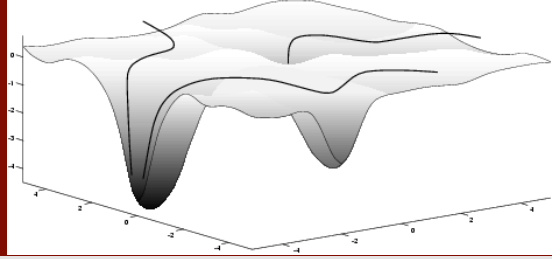
Evolution is steepest descent



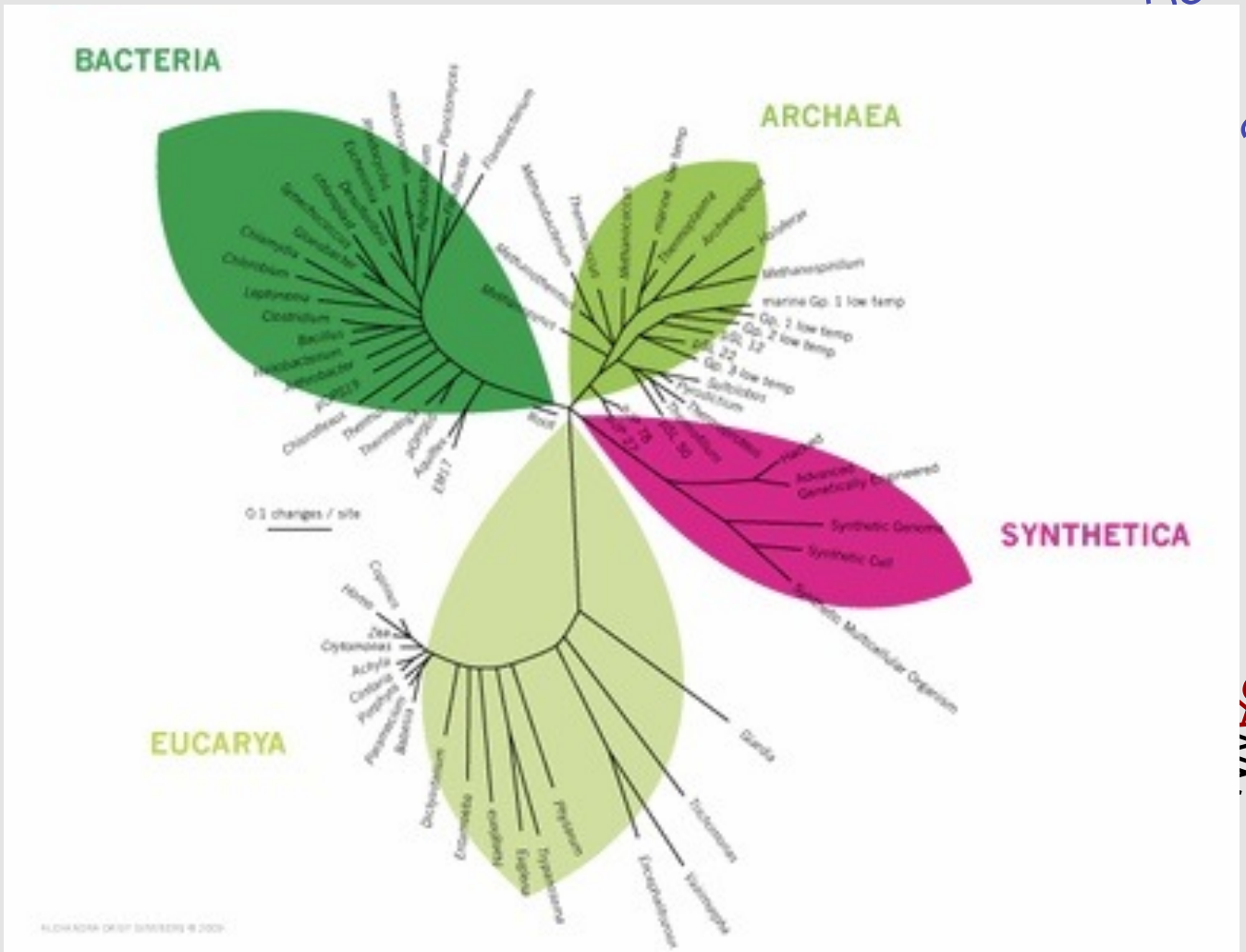


- Evolution can be expected to take the route of steepest descent
- The starting point determines (limits) the outcome
- This is analogous to Feyerabend's call for the development of many theories (starting points) no matter how poorly their initial ability to explain data

Discussion Points



- Biotechnology: Aligning the interest of the createe with the creator
- Bio-computation: What can we learn using sequencing approaches in combination with natural selection?
- Natural selection is iterative recombination: Equivalent to a pruned evaluation tree
- Most of the search space is inaccessible
- Should we explore earlier versions? (true new starting points)



Magnetic

GGCTAGCC

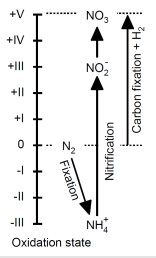
GGCTAGCC

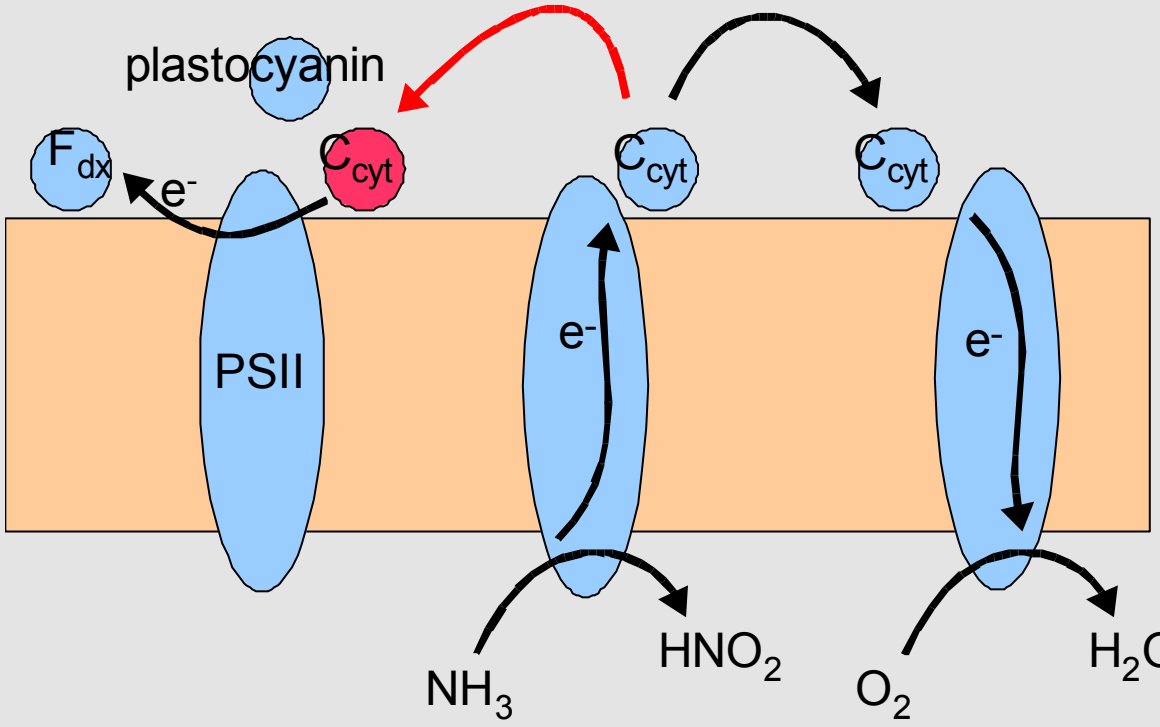
CCGATCGG
GGCTAGCC

GGCTAGCC

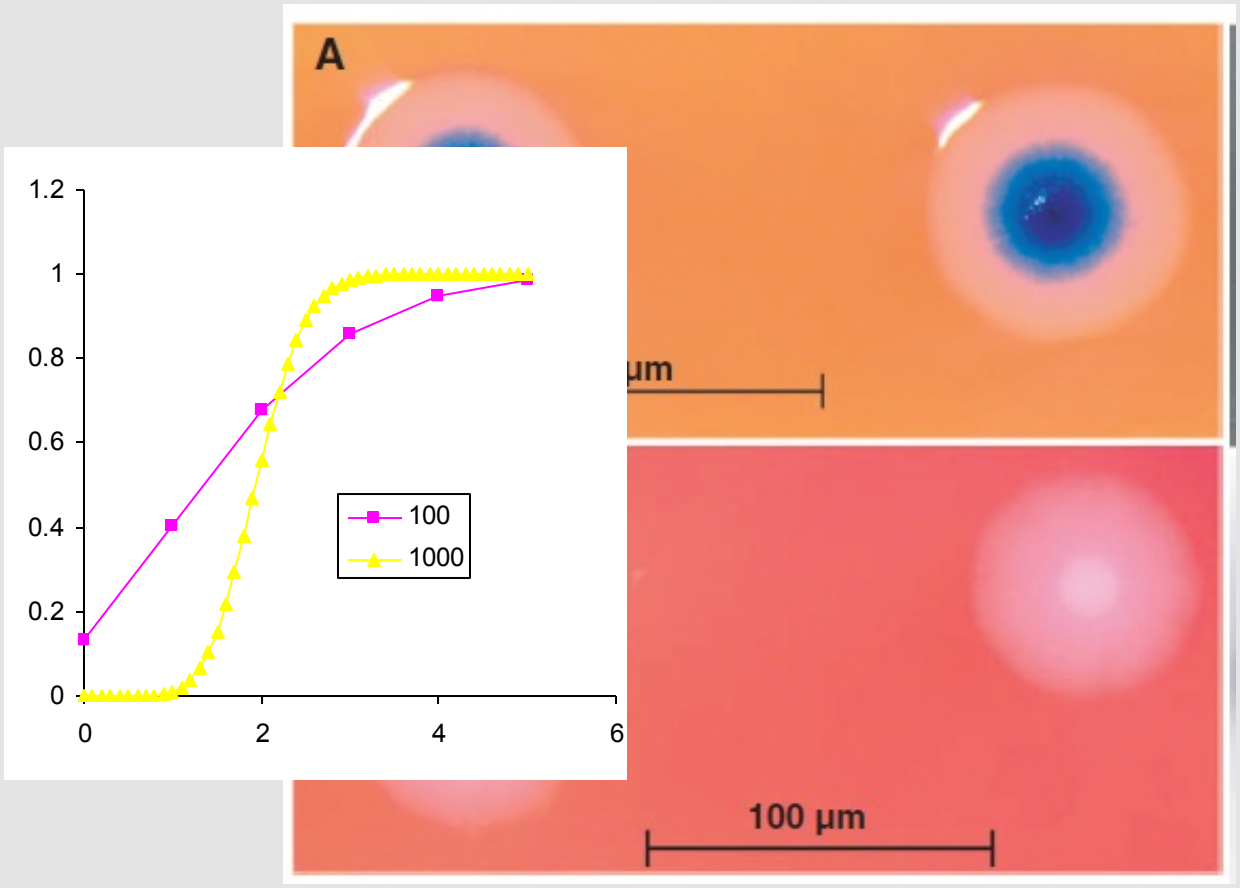
NNNNNNNNNNTGCATGCA

Potential rerouting of the electron chain





Plastocyanin and cytochrome C could exchange electrons



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