

## Microbial Taxonomy

Traditional taxonomy or the classification through **identification** and **nomenclature** of microbes, both "prokaryote" and eukaryote, has been in a mess – we were stuck with it for traditional reasons.

A "natural" taxonomy would be based on evolutionary relatedness: Thus, organisms in same "genus" (a collection of "species") would have similar properties in a fundamental sense.

A natural taxonomy of macrobes has long been possible: Large organisms have many easily distinguished features (e.g., body-plans and developmental processes, that can be used to describe hierarchies of relatedness).

Microbes usually have few distinguishing properties that relate them, so a hierarchical taxonomy mainly has not been possible.

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Recent advances in **molecular phylogeny** have changed this picture. We now have a relatively quantitative way to view **biodiversity**, in the context of phylogenetic maps or evolutionary trees.

Slowly evolving molecules (e.g. rRNA) used for large-scale structure; "fast- clock" molecules for fine-structure.

The literature language (e.g. "species") and formal nomenclature, however, remain solidly rooted in the tradition of Linnaeus at this time. (You have to call them something!)

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**Table 11.4** Some phenotypic characteristics of taxonomic value

Major category	Components
I. Morphology	Shape; size; Gram reaction; arrangement of flagella, if present
II. Motility	Motile by flagella; motile by gliding; motile by gas vesicles; nonmotile
III. Nutrition and Physiology	Mechanism of energy conservation (phototroph, chemoorganotroph, chemolithotroph); relationship to oxygen; temperature, pH, and salt requirements/tolerances; ability to use various carbon, nitrogen, and sulfur sources; growth factor requirements
IV. Other factors	Pigments; cell inclusions, or surface layers; pathogenicity; antibiotic sensitivity

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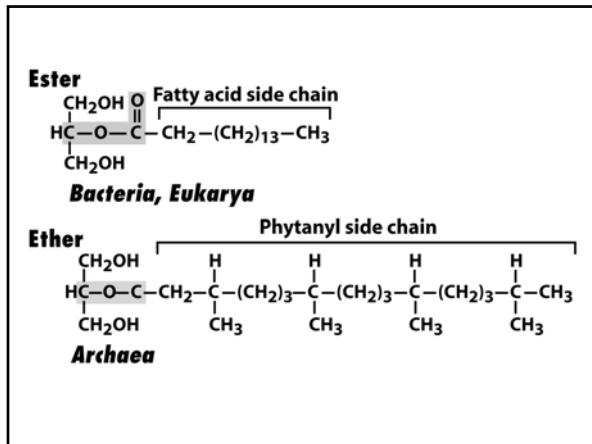
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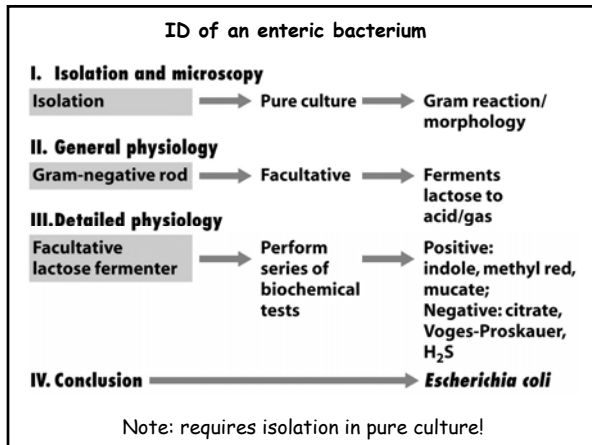
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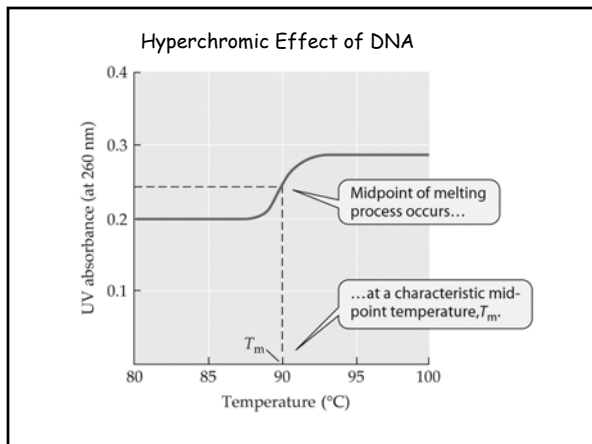
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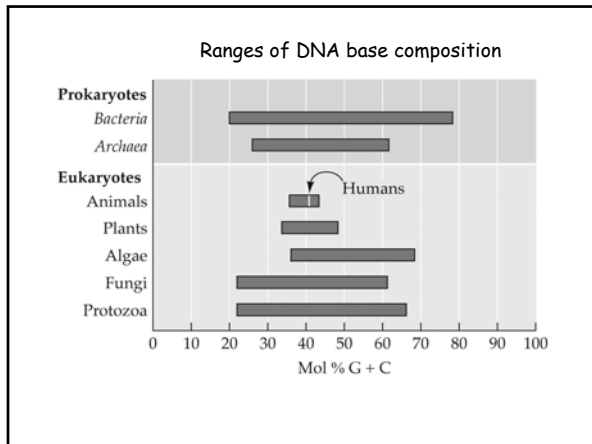
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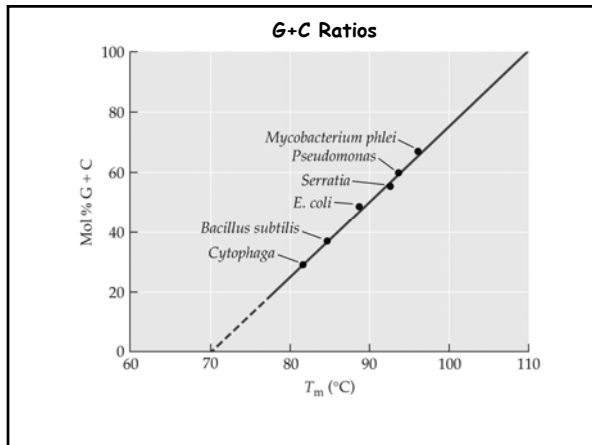
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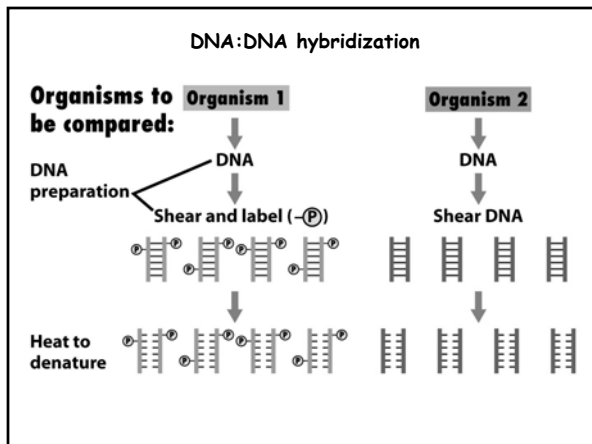
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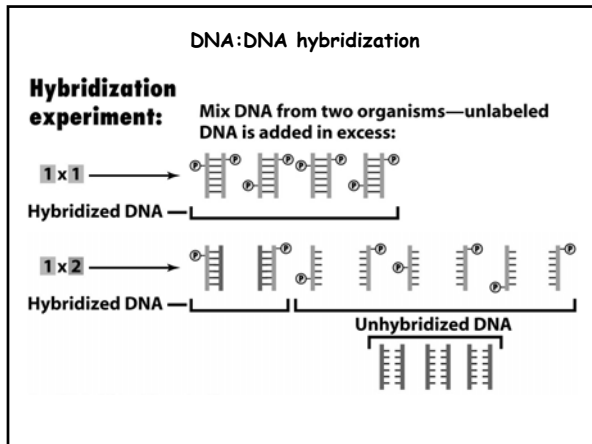
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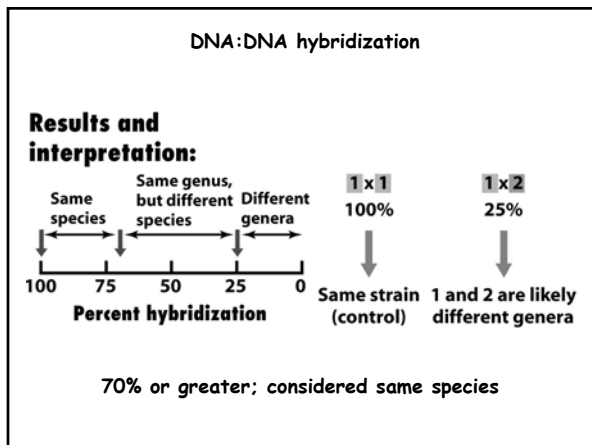
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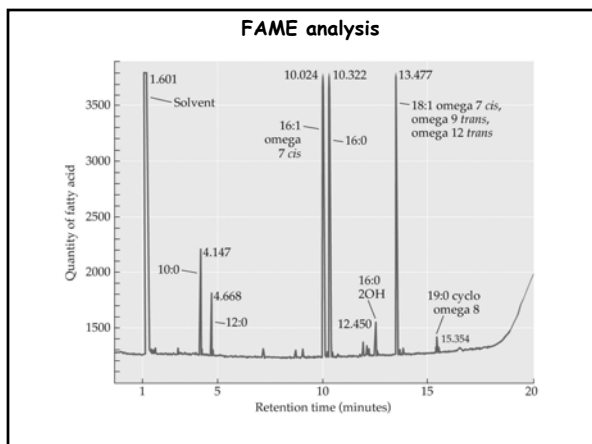
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**Classes of Fatty Acids in Bacteria**

Class/Example	Structure of example
I. <b>Saturated:</b> tetradecanoic acid	$\text{HO}-\text{C}(=\text{O})-(\text{CH}_2)_{12}-\text{CH}_3$
II. <b>Unsaturated:</b> <i>omega-7-cis</i> hexadecanoic acid	$\text{HO}-\text{C}(=\text{O})-(\text{CH}_2)_6-\text{C}(\text{H})=\text{C}(\text{H})-(\text{CH}_2)_6-\text{CH}_3$
III. <b>Cyclopropane:</b> <i>cis 7, 8 methylene</i> hexadecanoic acid	$\text{HO}-\text{C}(=\text{O})-(\text{CH}_2)_7-\text{C}(\text{H})-\text{C}(\text{H})-(\text{CH}_2)_5-\text{CH}_3$
IV. <b>Branched:</b> 13-methyltetradecanoic acid	$\text{HO}-\text{C}(=\text{O})-(\text{CH}_2)_{10}-\text{C}(\text{H})(\text{CH}_3)-\text{CH}_3$
V. <b>Hydroxy:</b> 3-hydroxytetradecanoic acid	$\text{HO}-\text{C}(=\text{O})-\text{CH}_2-\text{C}(\text{H})(\text{OH})-(\text{CH}_2)_{10}-\text{CH}_3$

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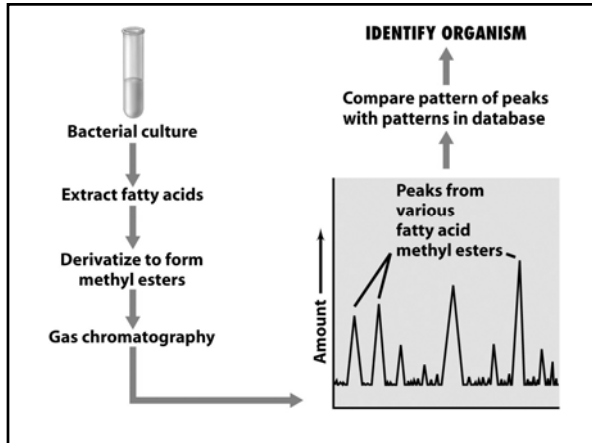
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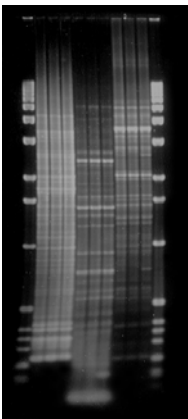
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**REP PCR Fingerprinting**

Lanes represent: Strains RL1, ES1, & ES2

- Three different types of PCR based genomic fingerprinting methods. Collectively known as **REP PCR**.
- Minimal genetic variability shown among three strains of iron-oxidizing bacteria.

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**Table 17.1** Hierarchical classification of the bacterium *Spirochaeta plicatilis*

Taxon	Name
Domain	<i>Bacteria</i>
Phylum	<i>Spirochaetes</i> (vernacular name: spirochetes)
Class	<i>Spirochaetes</i>
Order	<i>Spirochaetales</i>
Family	<i>Spirochaetaceae</i>
Genus	<i>Spirochaeta</i>
Species	<i>plicatilis</i>

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**Table 11.6** Taxonomic ranks and numbers of known prokaryotic species<sup>a</sup>

Rank	<i>Bacteria</i>	<i>Archaea</i>	Total
Domains	1	1	2
Phyla	25	4 <sup>a</sup>	29
Classes	34	9	43
Orders	78	13	91
Families	230	23	243
Genera	1227	79	1306
Species	6740	289	7029

<sup>a</sup>Numbers represent validly named genera and species of *Bacteria* and *Archaea* as of 2005. The phyla category for *Archaea* includes the Korarchaeota and the Nanoarchaeota, not yet officially recognized phyla.

Source: Garrity, G.M., Libum, T.G., and Bell, J.A. 2005. *Bergey's Manual of Systematic Bacteriology*, 2d ed., Vol. 2, part A, pp159-220. Springer-Verlag, New York.

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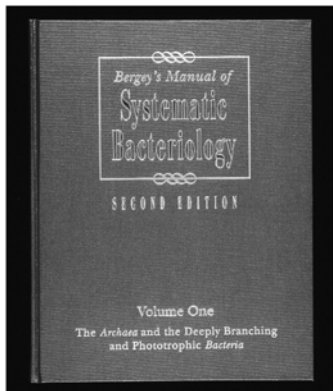
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### Taxonomy Summary

Classical physiological descriptions of microbes constitute a taxonomy, but do not provide relationships (except as might be inferred subjectively).

Methods such as G+C ratios, FAME, DNA-DNA hybridization, or REP PCR establish relationships, but only if close, i.e., they are not sufficiently general to be broadly applicable.

All these methods require pure-cultivation of organisms for characterization, but we can't cultivate much of what is out there.

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### Importance of a Molecular Biological Approach

- **Traditional culturing** techniques isolate ~1% of the total bacteria in marine ecosystems, thereby severely underestimating diversity and community structure.
- Because nutrient-rich **culture media** have been historically used during enrichment procedures, bacteria which may be dominant in natural communities are selected against in favor of copiotrophic (weedy) bacteria.
- **SSU rRNAs** and their respective genes are excellent descriptors of microbial taxa based on phylogeny.

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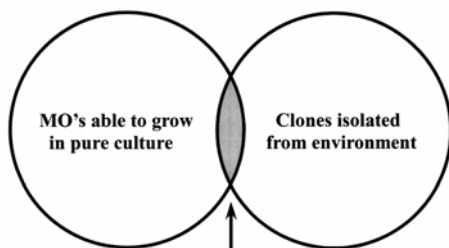
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>1% Crossover  
between these groups

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## Regarding Molecular Phylogeny

**The Root of the Problem:** Unlike zoology and botany, microbiology developed without the knowledge of phylogenetic relationships among the organisms studied.

- Milestone #1: Zuckerkandl and Pauling (1965) "Semantides" (i.e., molecules as documents of evolutionary history).
- Milestone #2: Pace (1986) Applied phylogeny concept to microbial ecology's need to take a census.
- Milestone #3: Woese (1987) Applied phylogeny concept to redefine microbial systematics or the need to understand microbial genealogy.

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*... the general course of evolution [for bacteria] will probably never be known, and there is simply not enough objective evidence to base their classification on phylogenetic grounds... For these and other reasons, most modern taxonomists have explicitly abandoned the phylogenetic approach.*

(Stanier *et al.*, 1976)

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## Why ribosomal RNAs?

- Found among all living organisms (for 3.8 of the last 4.5 billion years). Integral part of protein synthesis machinery.
- Cell component analyses provide culture-independent means of investigating questions in microbial ecology (lack of morphology).
- rRNAs offer a type of sequence information that makes them excellent descriptors of an organism's evolutionary history.
- No detectable horizontal gene transfer, especially important for the prokaryotes.
- Large and growing database; RDP contains ~100K SSU rRNAs.

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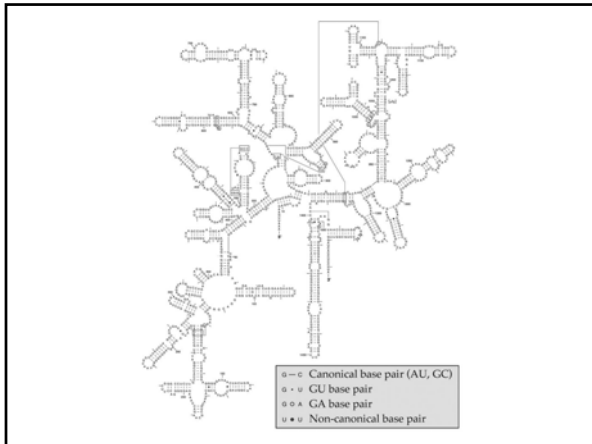
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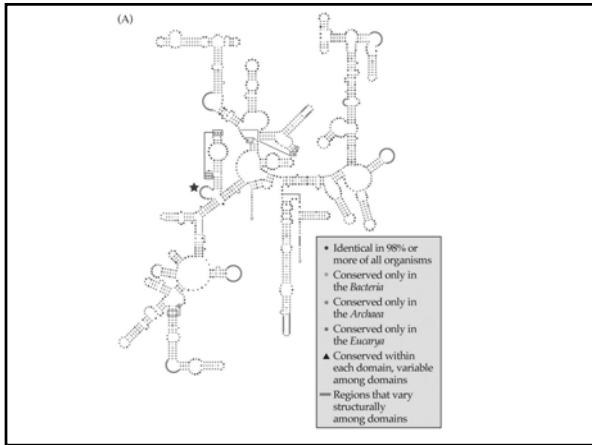
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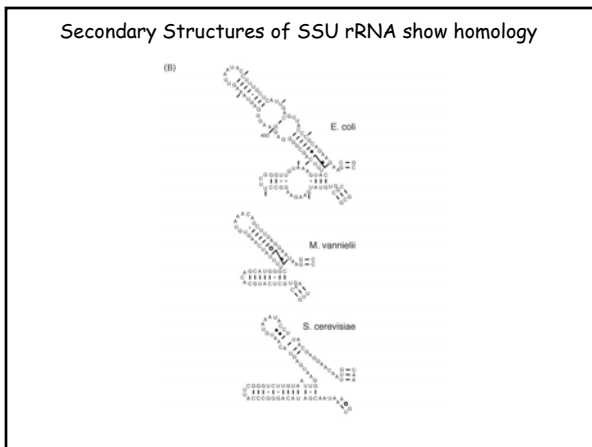
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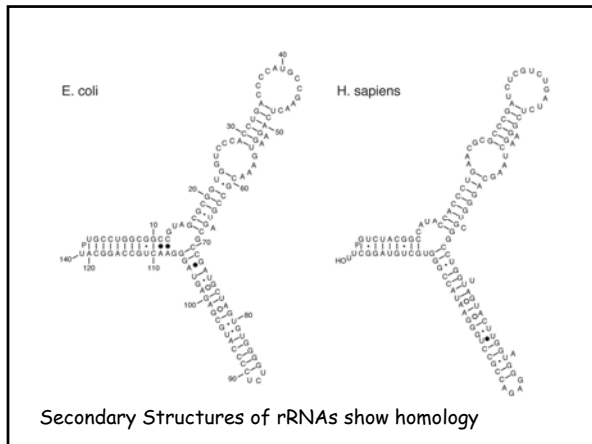
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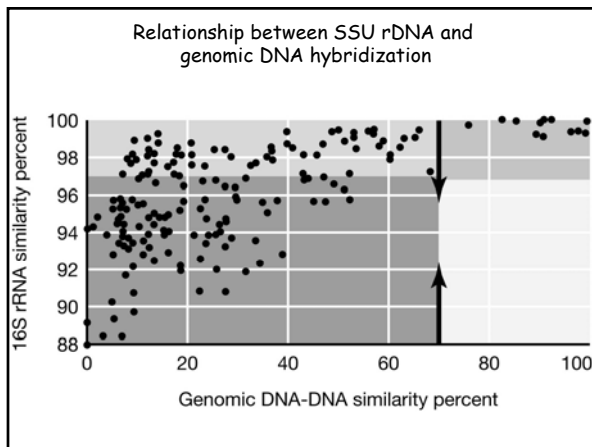
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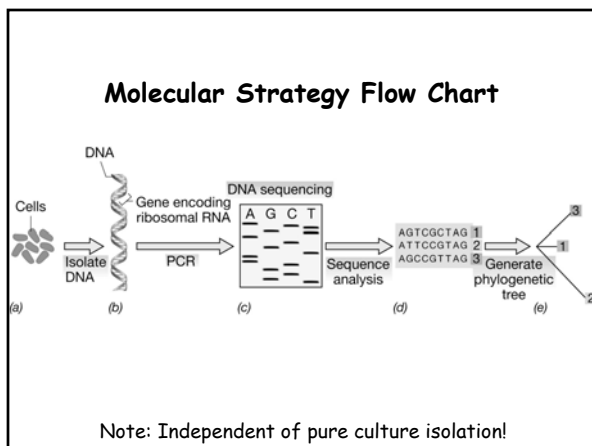
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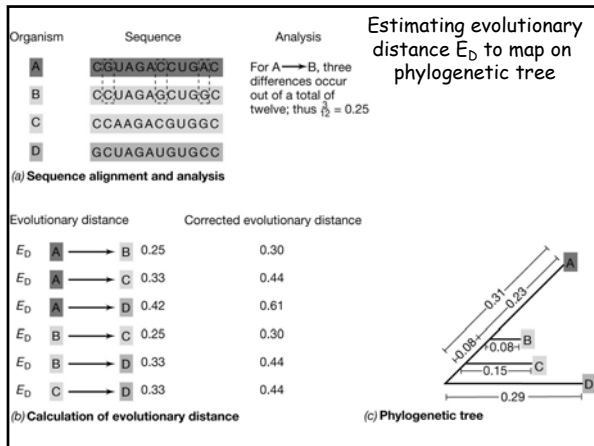
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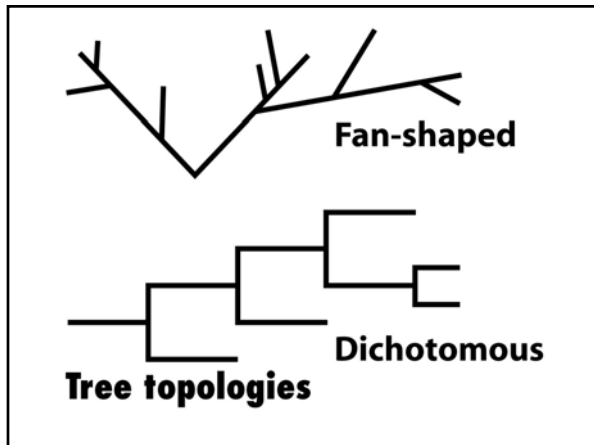
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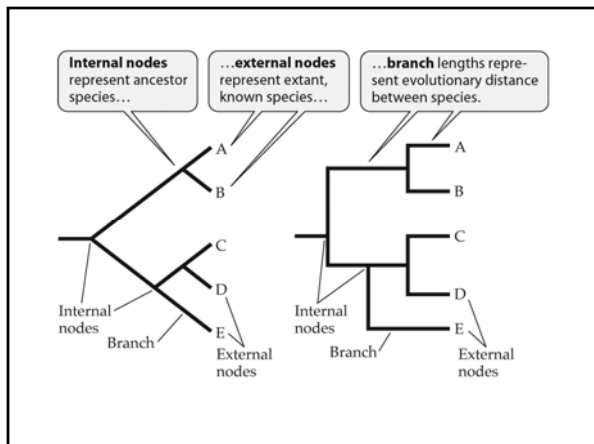
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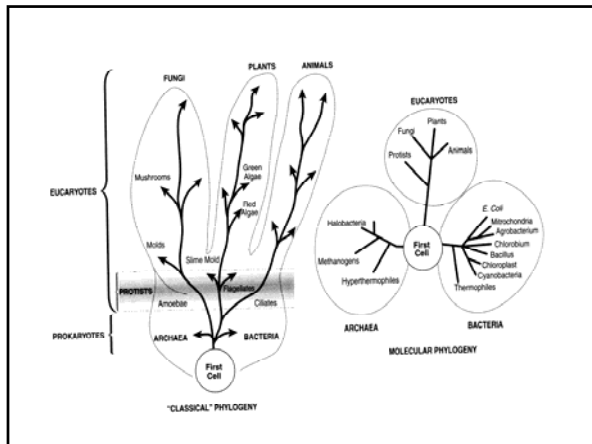
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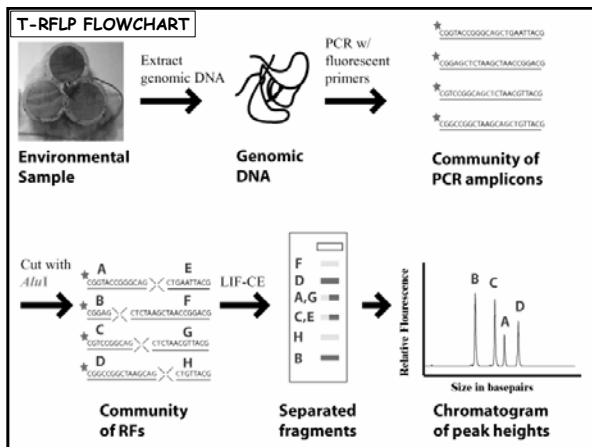
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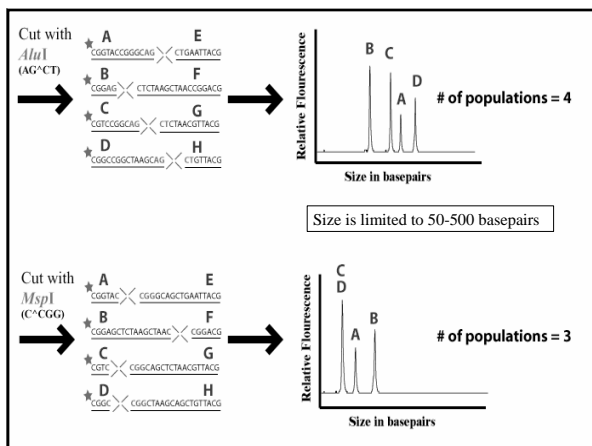
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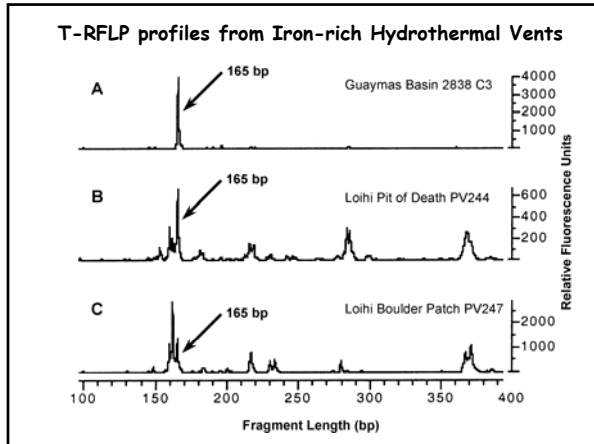
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### T-RFLP profiles from Iron-rich Hydrothermal Vents




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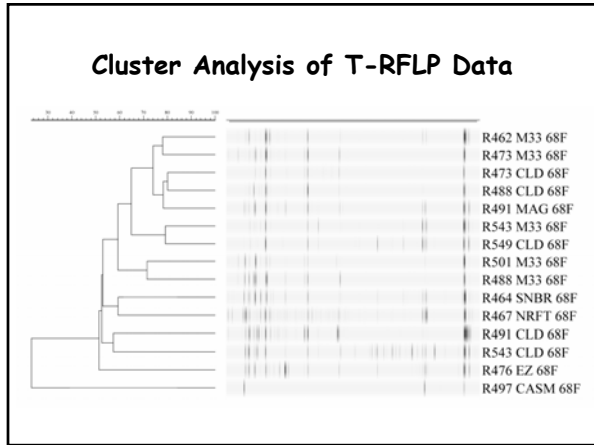
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### Cluster Analysis of T-RFLP Data




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**Table 11.1** Signature sequences from 16S or 18S rRNA defining the three domains of life

Oligonucleotide signatures <sup>a</sup>	Approximate position <sup>b</sup>	Occurrence among <sup>c</sup>		
		Archaea	Bacteria	Eukarya
CACYYG	315	0	>95	0
AAACUAAA	910	3	100	0
AAACUAAAG	910	100	0	100
YUYAAUUG	960	100	<1	100
CAACCYCR	1110	0	>95	0
UCCUG	1380	>95	0	100
UACACACCG	1400	0	>99	100
CACACACCG	1400	100	0	0

<sup>a</sup> Y, any pyrimidine; R, any purine.

<sup>b</sup> Refer to Figure 11.11c for numbering scheme of 16S rRNA.

<sup>c</sup> Occurrence refers to percentage of organisms examined in any domain that contain that sequence.

Signature sequences can be obtained at any level of taxonomic hierarchy

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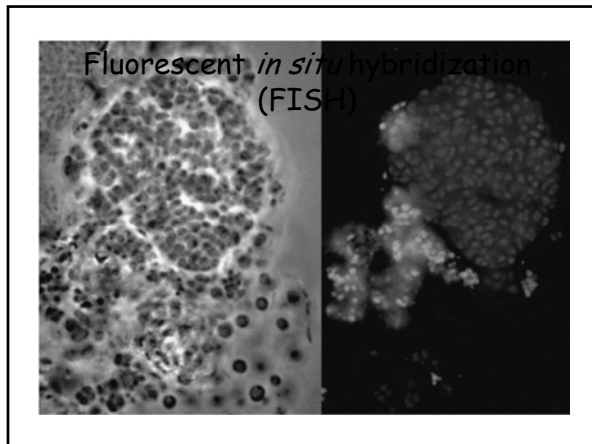
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### Take Home Message

- Phylogeny is right or wrong, we try to infer it the best we can.
- Taxonomy is useful or not, depending upon your point of view.
- Phylogeny allows us to ask testable questions, e.g., hypothesis testing.
  - microbial ecology relationships can now be truly examined
  - relationships between MOs and their genes can be studied
  - infer dynamics of sequence change (Rolex vs Timex)

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**Table 11.3 Summary of major differential features among Bacteria, Archaea, and Eukarya<sup>a</sup>**

Characteristic	Bacteria	Archaea	Eukarya
<b>Morphological and Genetic</b>			
Prokaryotic cell structure	Yes	Yes	No
DNA present in covalently closed and circular form	Yes	Yes	No
Histone proteins present	No	Yes	Yes
Membrane-enclosed nucleus	Absent	Absent	Present
Cell wall	Muramic acid present	Muramic acid absent	Muramic acid absent
<b>Membrane lipids</b>			
Ribosomes (mass)	70S	70S	80S
Initiator tRNA	Formylmethionine	Methionine	Methionine
Inserts in most genes	No	No	Yes
Operons	Yes	Yes	No
Capping and poly-A tailing of mRNA	No	No	Yes
Flamands	Yes	Yes	Rare
Ribosome sensitivity to diphtheria toxin	No	Yes	Yes
RNA polymerases (see Figure 11.19)	One (4 subunits)	Several (8-12 subunits each)	Three (12-14 subunits each)
Transcription factors required (see Section 7.11)	No	Yes	Yes
Promoter structure (see Sections 7.10 and 7.11)	-10 and -35 sequences (Pribnow box)	TATA box	TATA box
Sensitivity to chloramphenicol, streptomycin, and kanamycin	Yes	No	No

<sup>a</sup> Note that for many features only particular representatives within a domain show the property.  
<sup>b</sup> Environmental genomics studies of prokaryotes in marine waters strongly suggest that nitrifying Archaea exist (see Section 18.6).

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**Table 11.3 Summary of major differential features among Bacteria, Archaea, and Eukarya<sup>a</sup>**

Characteristic	Bacteria	Archaea	Eukarya
<b>Physiological/Special Structures</b>			
Methanogenesis	No	Yes	No
Dissimilative reduction of S <sup>0</sup> or SO <sub>4</sub> <sup>2-</sup> to H <sub>2</sub> S, or Fe <sup>3+</sup> to Fe <sup>2+</sup>	Yes	Yes	No
Nitrification	Yes	No <sup>b</sup>	No
Denitrification	Yes	Yes	No
Nitrogen fixation	Yes	Yes	No
Chlorophyll-based photosynthesis	Yes	No	Yes (in chloroplasts)
Rhodospin-based energy metabolism	Yes	Yes	No
Chemolithotrophy (Fe, S, H <sub>2</sub> )	Yes	Yes	No
Gas vesicles	Yes	Yes	No
Synthesis of carbon storage granules composed of poly-β-hydroxyalkanoates	Yes	Yes	No
Growth above 80° C	Yes	Yes	No
Growth above 100° C	No	Yes	No

<sup>a</sup> Note that for many features only particular representatives within a domain show the property.  
<sup>b</sup> Environmental genomic studies of prokaryotes in marine waters strongly suggest that nitrifying Archaea exist (OPD/Section 18.6).

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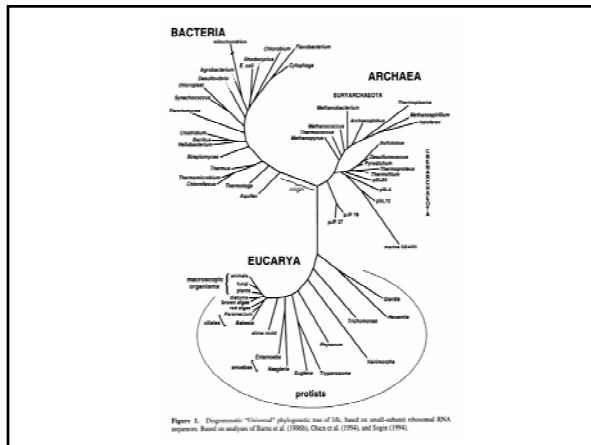
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**Some Lessons from the BIG TREE: Map of the Biological Record**

Single origin for all life on Earth...

- Central Dogma intact.
- ATP and PMF are universal themes.
- Uniformity among chiral carbon compounds (sugars & AAs).
- Hot start origin...

General topology implies:

- Three "primary lines of evolutionary descent."
- The Eucarya "nuclear" lineage almost as old as other two.
- Prokaryotes split between *Bacteria* and *Archaea*.
- Shown for only a limited number of representative org's.
- Mitochondria and chloroplasts proven to be of bacterial origin.

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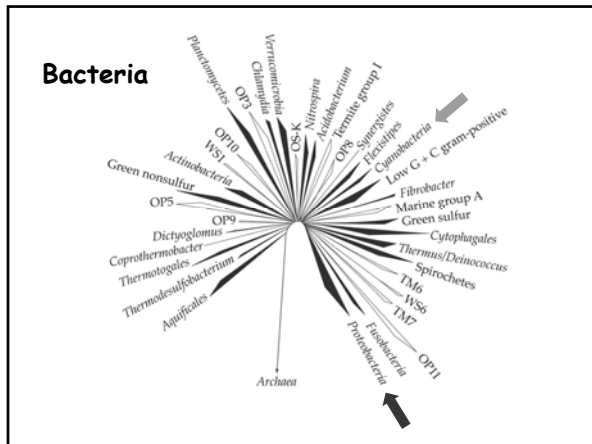
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**Some Lessons from the BIG TREE: Map of the Biological Record**

Evolutionary "clock" is NOT constant between different lineages

- Terminal nodes NOT all the same length, so not constant for all organisms either!
- Endosymbionts sped up very fast (semi-autonomous)
- Eucarya – Fast clocks
- Archaea – Slow clocks
- Bacteria – Intermediate

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**Horizontal gene transfer**

This lateral flow of information across microbial taxa occurs via the transfer of genes by:

**conjugation, transduction, and transformation.**

Rem: These are one-way processes!

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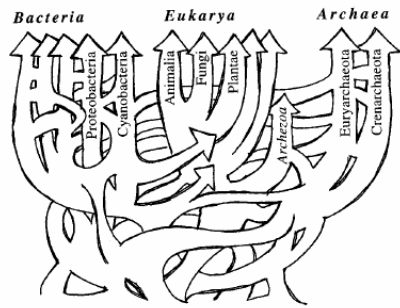
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Fig. 3. A reticulated tree, or net, which might more appropriately represent life's history.



Doolittle's Universal Tree (1999)

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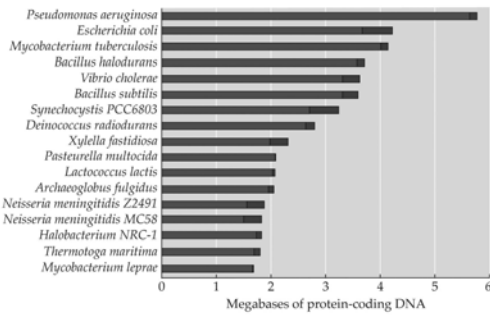
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### Relative importance of horizontal gene transfer




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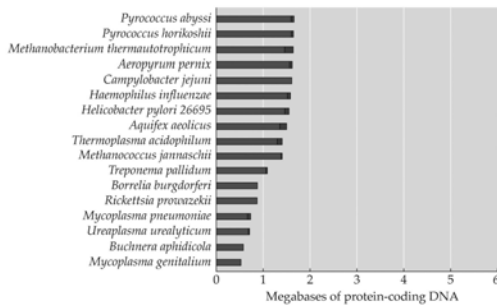
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### Relative importance of horizontal gene transfer




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**Some Lessons from the BIG TREE: Map of the Biological Record**

What does genome sequencing and study of functional genomics add to our perspective?

- The central information processing machinery encompasses core genome.
- Metabolic functions, that's when relationships get murky.
- Endosymbiosis involves more than simply organelles, i.e., two-way transfer of genes with most going to the nucleus.
- Mitochondria have been at it much longer than chloroplasts.

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**A Bit on the Evolution of Evolutionary Thought**

A. Prior to the late 19th century, the concept of evolution was on the **evolutionary ladder**. Thus, we still deal in "higher and lower" eucaryotes (I try not to use these terms – they are dumb), "missing links," and "primitive" organisms.

B. In its milieu, *E. coli* is as highly evolved as are we. *E. coli* is **simple** (~5x10<sup>9</sup> bp genome), we are **complex** (~3x10<sup>9</sup> bps); complexity has nothing to do with *evolutionary advancement*.

C. Lineages evolve by diversification, not *progression*. !!!

D. There is no such thing as a *primitive* organism alive today. **Simple**, yes, but still a finely honed product of ~ 4 billion years under the selective hammer of the niches that it and its progenitors have occupied.

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**C-value paradox:  
Organism complexity  
does not correlate to  
genome size**

**TABLE 13.3 C values from eukaryotic organisms ranked by size**

Species	C value (kb)
<i>Nannicola pellucidula</i> (diatom)	35,000
<i>Drosophila melanogaster</i> (fruitfly)	180,000
<i>Paramecium aurelia</i> (ciliate)	190,000
<i>Gallus domesticus</i> (chicken)	1,200,000
<i>Ergasilpha cichoracearum</i> (fungus)	1,500,000
<i>Cyprinus carpio</i> (carp)	1,700,000
<i>Lampetra planeri</i> (lamprey)	1,900,000
<i>Bos constrictor</i> (snake)	2,100,000
<i>Parascaris equorum</i> (roundworm)	2,500,000
<i>Carcharias obscurus</i> (shark)	2,700,000
<i>Rattus norvegicus</i> (rat)	2,900,000
<i>Xenopus laevis</i> (toad)	3,100,000
<b>Homo sapiens (human)</b>	<b>3,400,000</b>
<i>Nicotiana tabacum</i> (tobacco)	3,800,000
<i>Paramecium caudatum</i> (ciliate)	8,600,000
<i>Schistocerca gregaria</i> (locust)	9,300,000
<i>Allium cepa</i> (onion)	18,000,000
<i>Coscinodiscus asteromphalus</i> (diatom)	25,000,000
<i>Lilium formosanum</i> (lily)	36,000,000
<i>Pinus resinosa</i> (pine)	68,000,000
<i>Amphiuma means</i> (newt)	84,000,000
<i>Protoperus arthipiscus</i> (lungfish)	140,000,000
<i>Ophioglossum petiolatum</i> (fern)	160,000,000
<i>Amoeba proteus</i> (amoeba)	290,000,000
<b>Amoeba dubia</b> (amoeba)	<b>670,000,000</b>

Compiled by Li and Graur (1991) from Cavalier-Smith (1985), Sparrow et al. (1972), and other references. The C value for humans is highlighted for reference.

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**Table 17.2** Comparison of *E. coli* and its primate host species<sup>a</sup>

Property	<i>E. coli</i>	<i>Homo sapiens</i>	Primates
Mol % G + C	48–52	42	42 <sup>b</sup>
16S–18S rRNA variability	>15 bases	?	<16 <sup>c</sup>
DNA/DNA reassociation	>70%	98.6% <sup>d</sup>	>70% <sup>e</sup>

<sup>a</sup>Adapted from J. T. Staley, *ASM News*, 1999.

<sup>b</sup>Value for all primates.

<sup>c</sup>Mouse 18S rRNA differs from humans by 16 bases.

<sup>d</sup>Comparison between *Homo sapiens* and chimpanzee.

<sup>e</sup>Comparison between *Homo sapiens* and lemurs.

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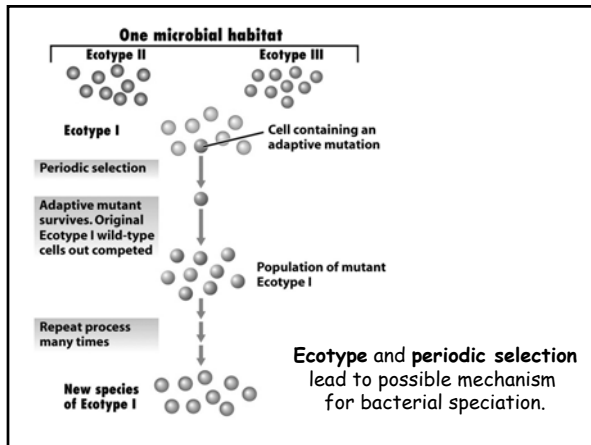
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