
Classifying microbes:
How do we sort all this out?

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.

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.

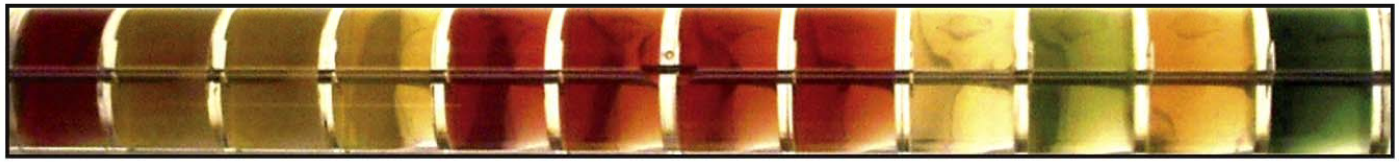
Slowly evolving molecules (e.g. rRNA) used for large-scale structure; "fast- clock" molecules for fine-structure.

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!)

Table 11.4 Some phenotypic characteristics of taxonomic value

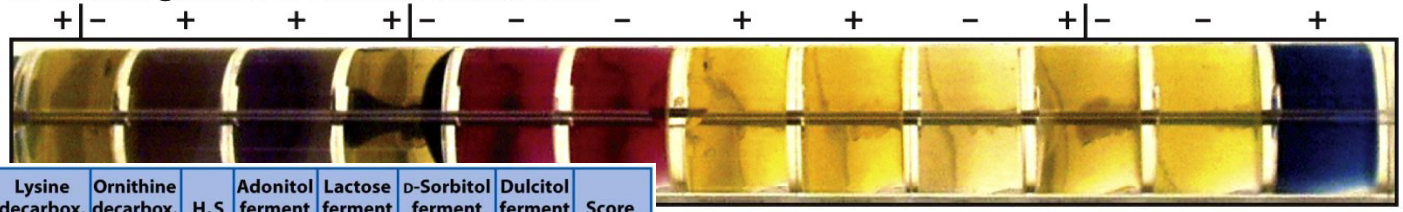
Major category	Components
I. Morphology	Shape; size; Gram reaction; arrangement of flagella, if present
II. Motility	Motile by flagella; motile by gliding; motile by gas vesicles; nonmotile
III. Nutrition and Physiology	Mechanism of energy conservation (phototroph, chemoorganotroph, chemolithotroph); 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

Enterotube™ reactions



Uninoculated Enterotube™

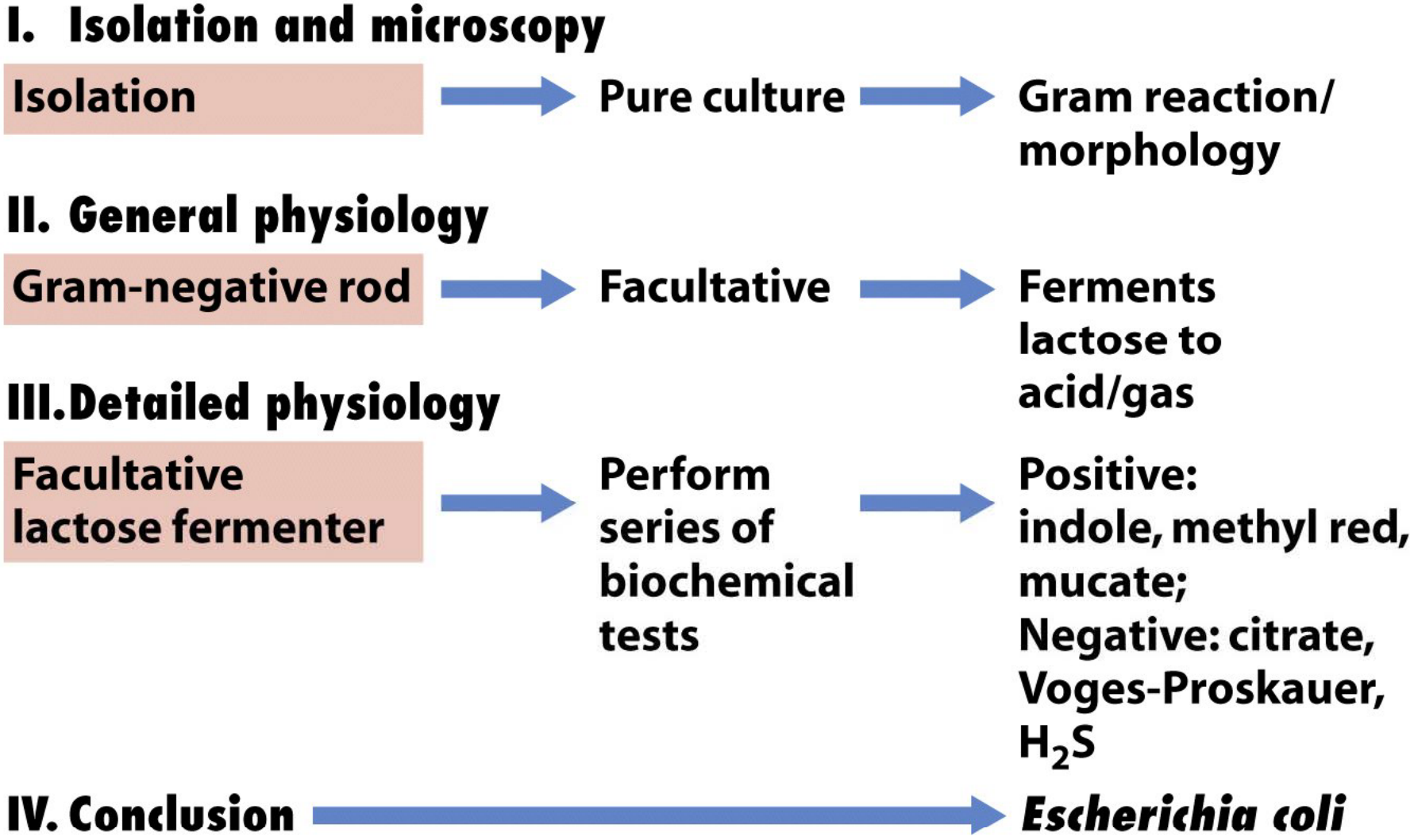
After 24h growth of *Salmonella enterica*



Species	D-Glucose acid	Lysine decarbox.	Ornithine decarbox.	H_2S	Adonitol ferment	Lactose ferment	D-Sorbitol ferment	Dulcitol ferment	Score
Test Results:	+	+	+	+	–	–	+	–	
Salmonella enterica	0.99	0.98	0.97	0.95	0.01	0.01	0.95	0.96	3.3 E-2
Proteus mirabilis	0.99	0.01	0.99	0.98	0.01	0.02	0.01	0.01	9.2 E-5
Yersinia enterocolitica	0.99	0.01	0.95	0.01	0.01	0.05	0.99	0.01	8.7 E-5
Enterobacter aerogenes	0.99	0.98	0.98	0.01	0.98	0.95	0.99	0.05	8.9 E-6
Klebsiella pneumoniae	0.99	0.98	0.01	0.01	0.90	0.98	0.99	0.30	1.3 E-7

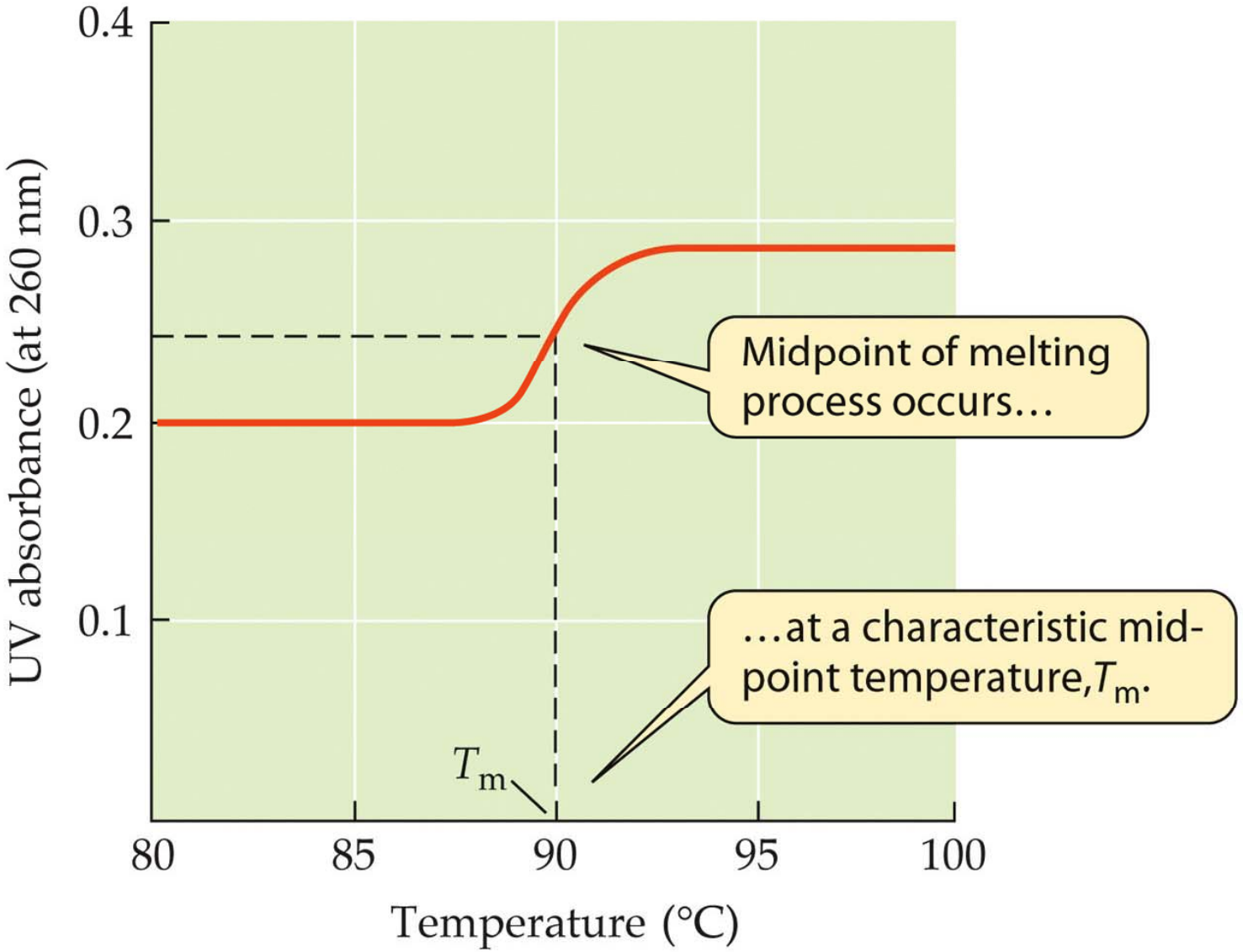
Figure 17.19b Microbiology: An Evolving Science
Joan Slonczewski and BD Diagnostics

ID of an enteric bacterium

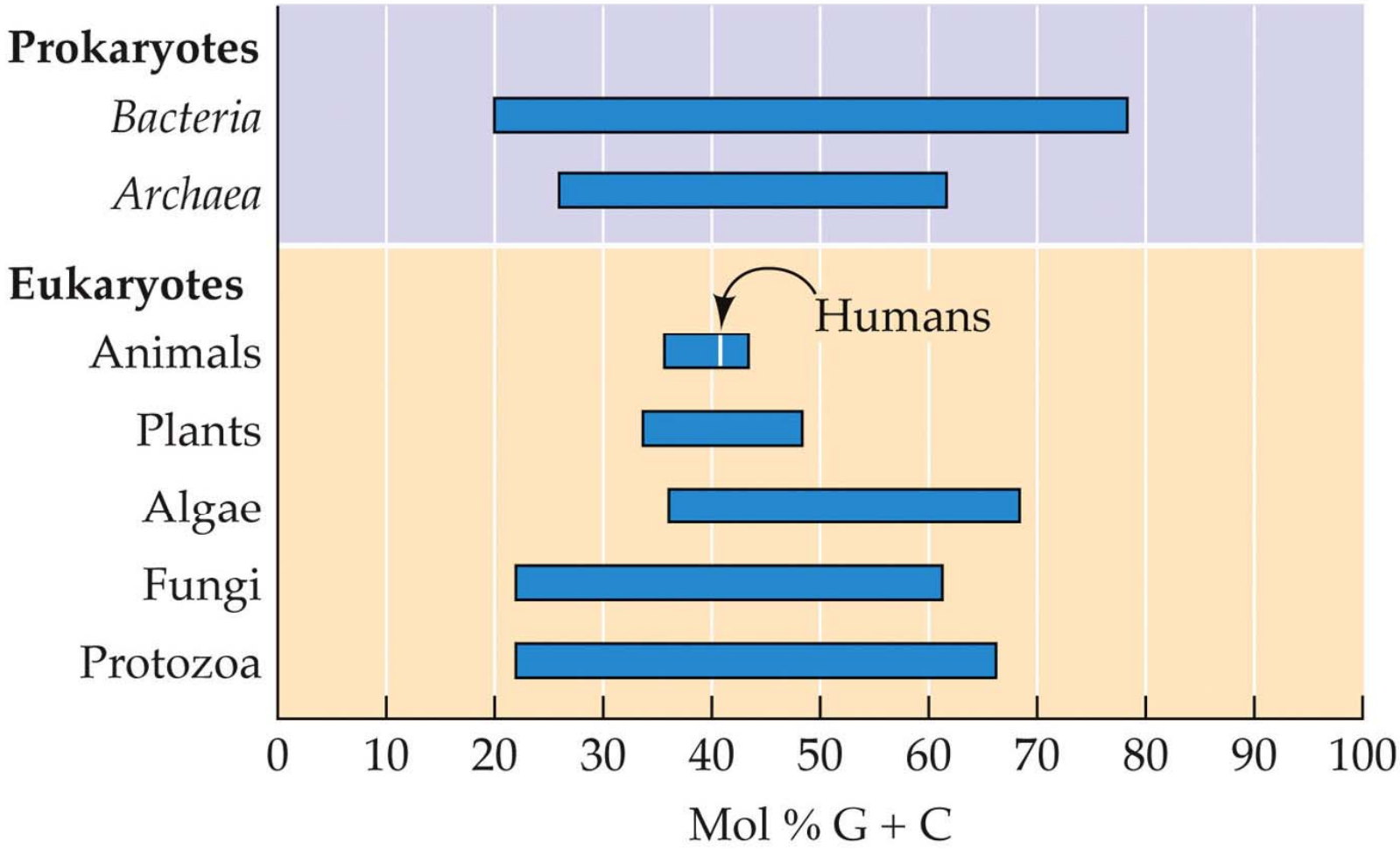


Note: requires isolation in pure culture!

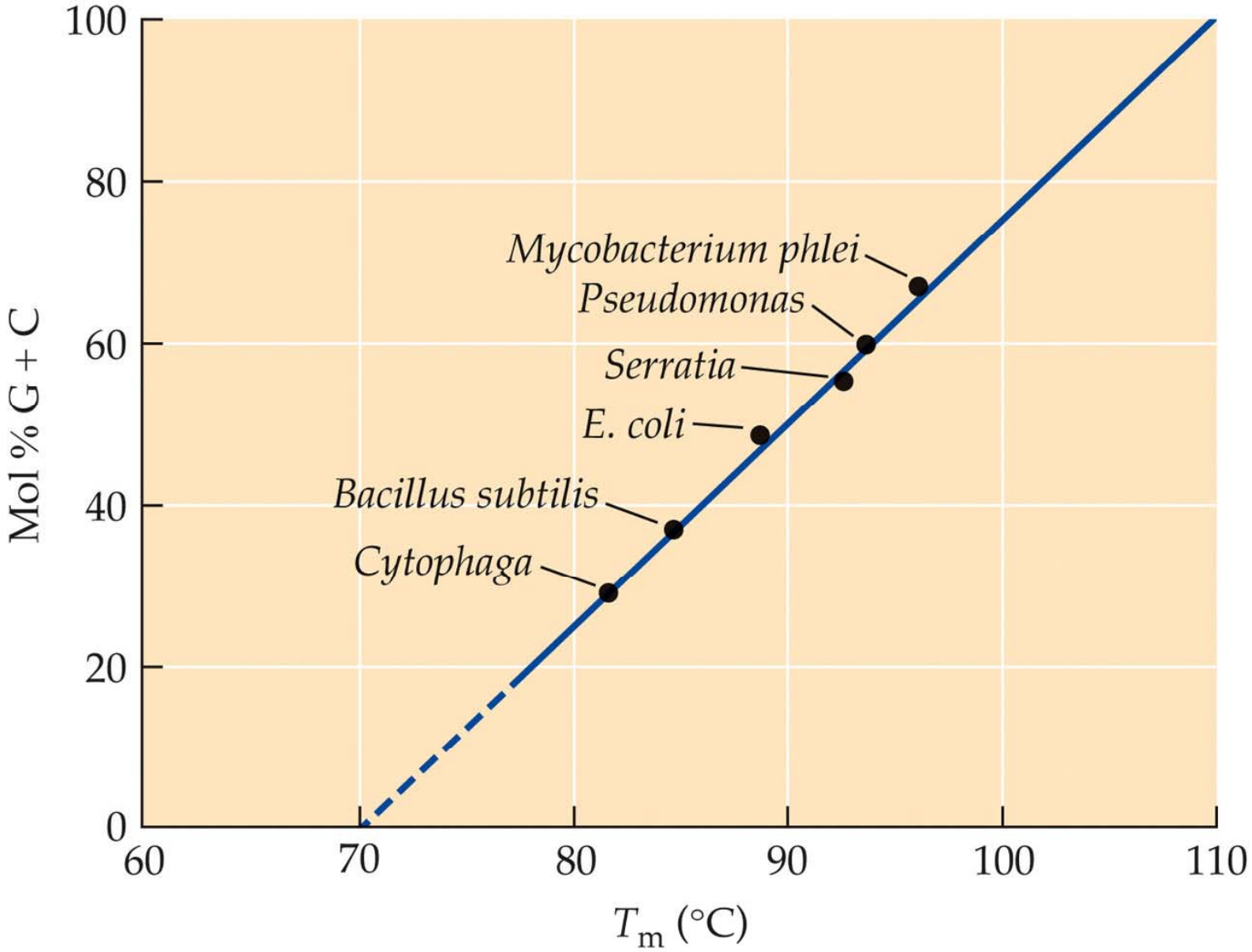
Hyperchromic Effect of DNA



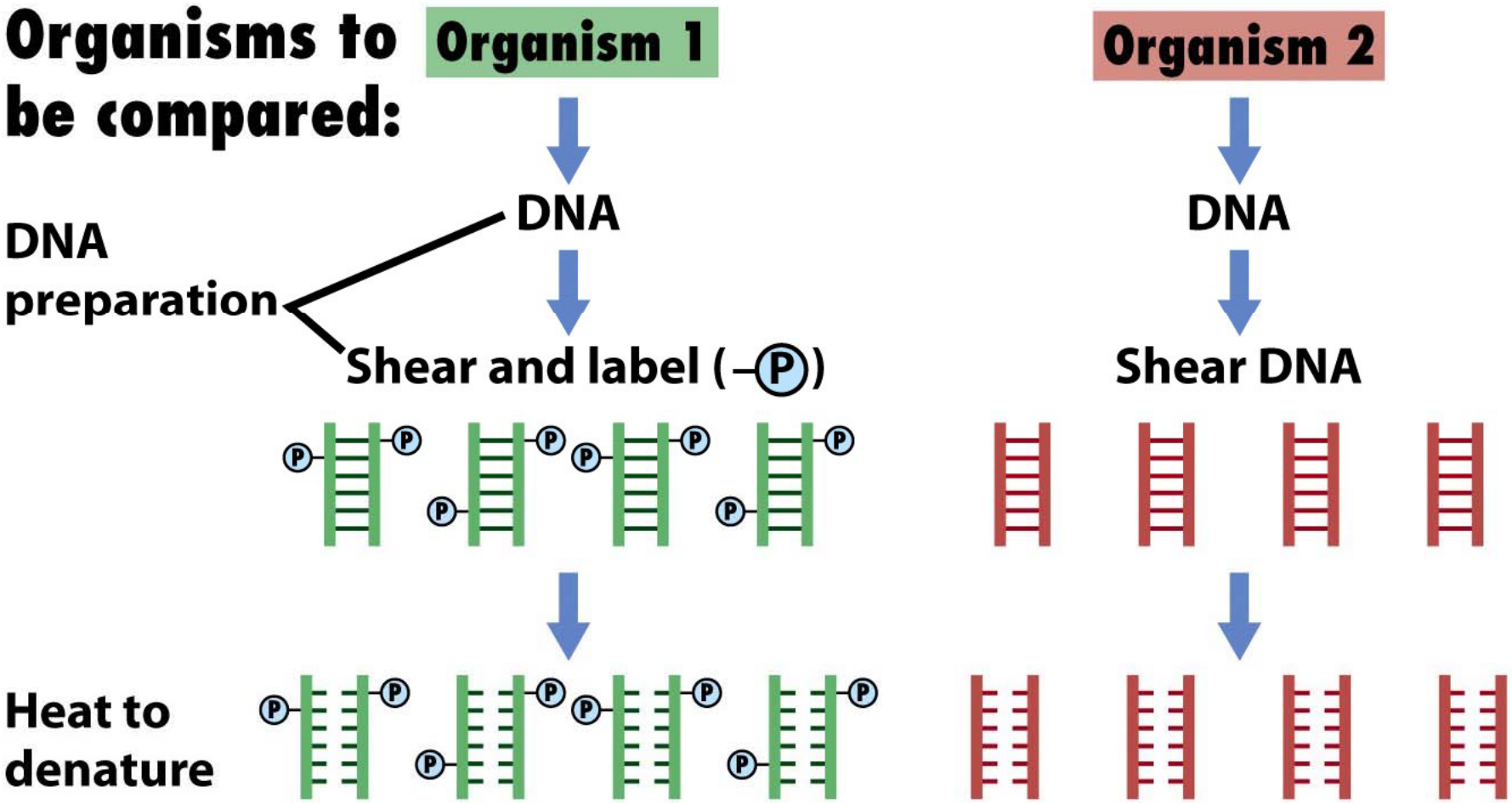
Ranges of DNA base composition



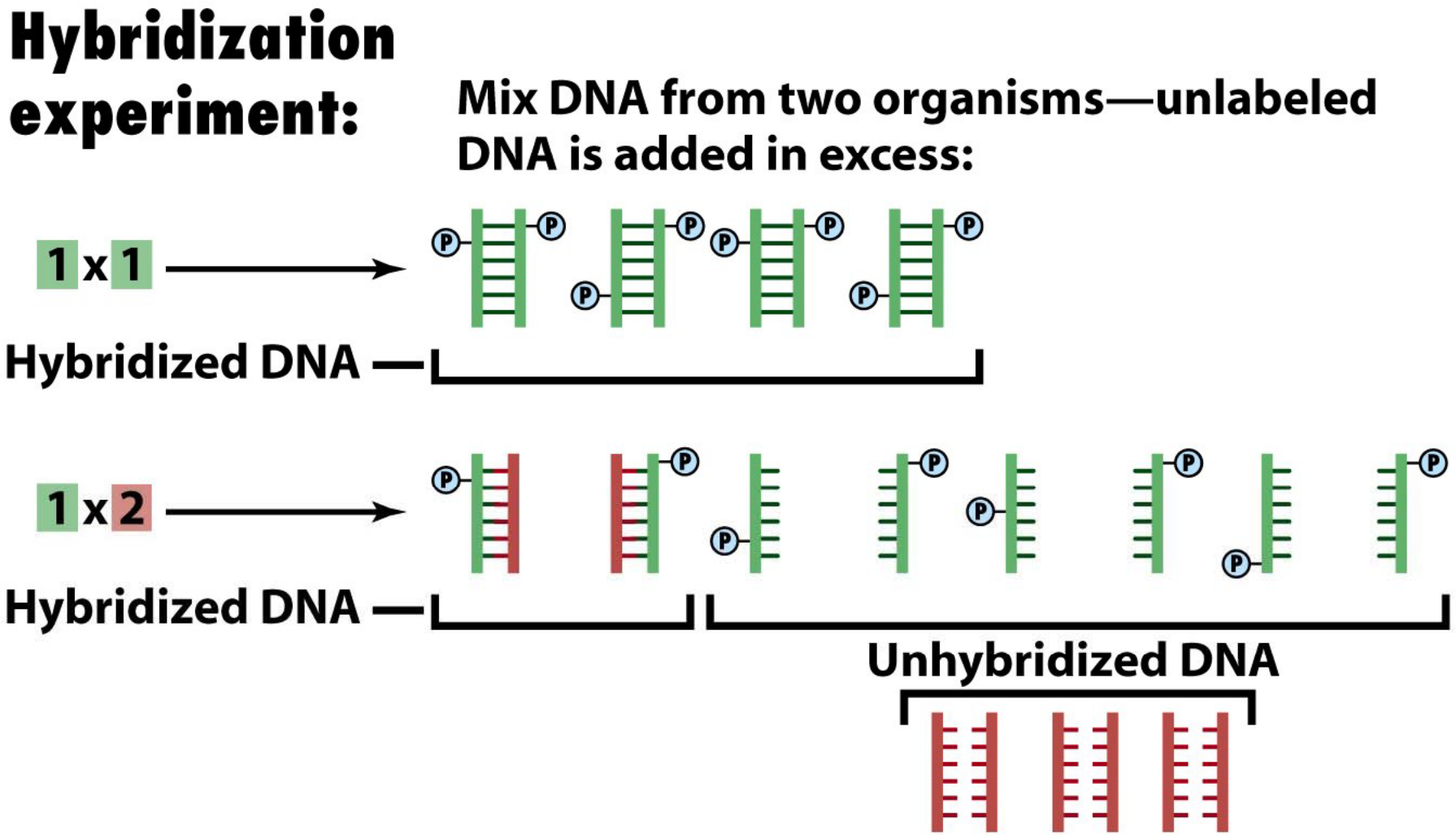
G+C Ratios


100
80
60
40
20
0
Mol % G + C
60
70
80
90
100
110
T_m (°C)
Mycobacterium phlei
Pseudomonas
Serratia
E. coli
Bacillus subtilis
Cytophaga

DNA:DNA hybridization

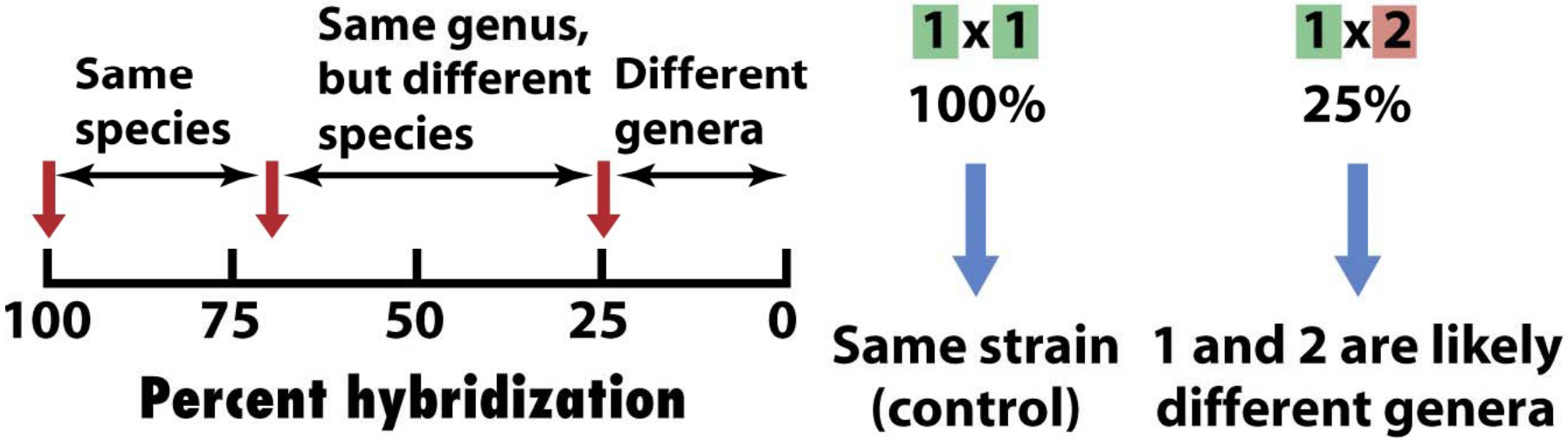


DNA:DNA hybridization



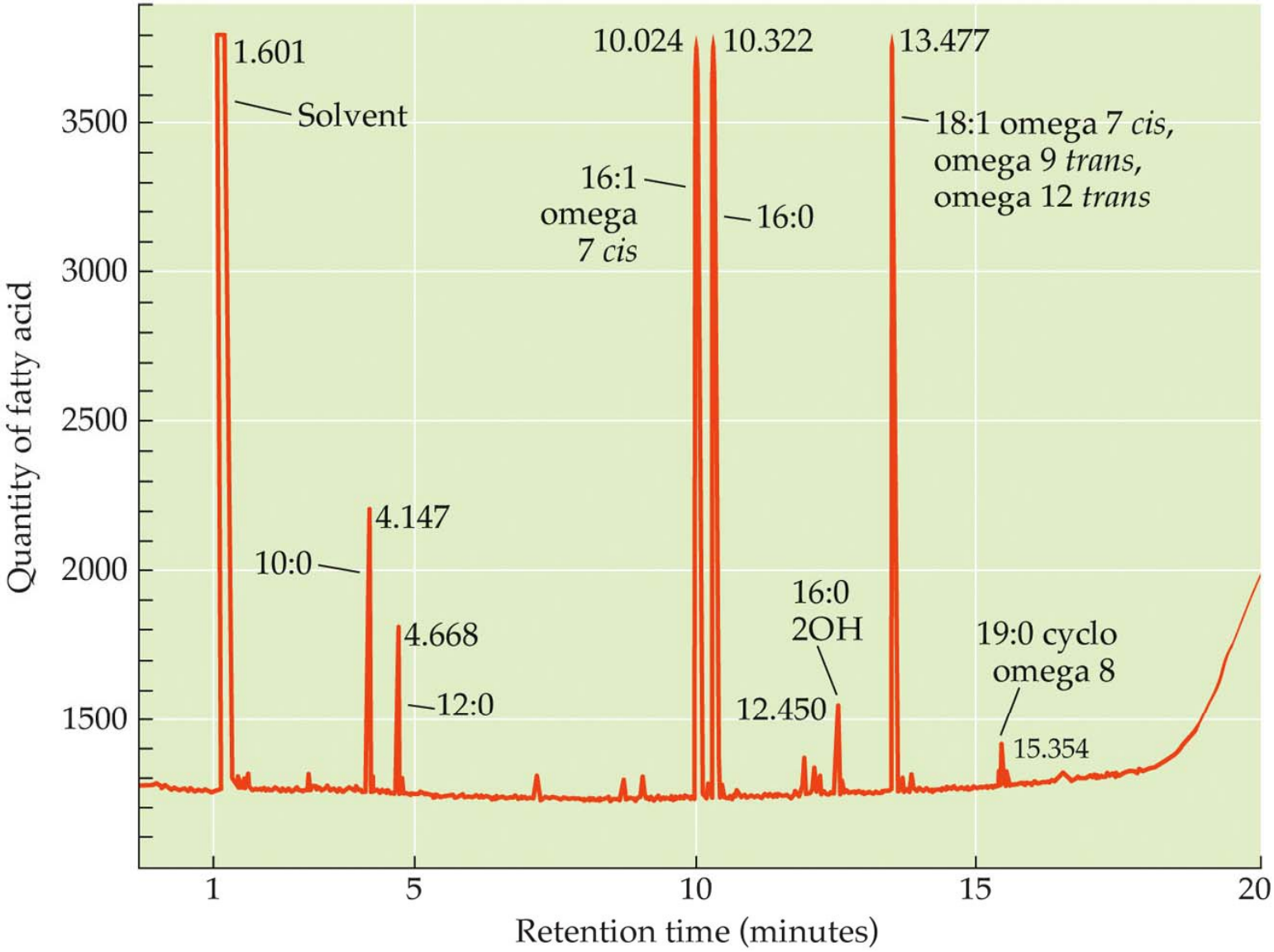
DNA:DNA hybridization

Results and interpretation:

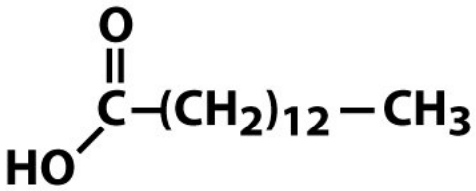
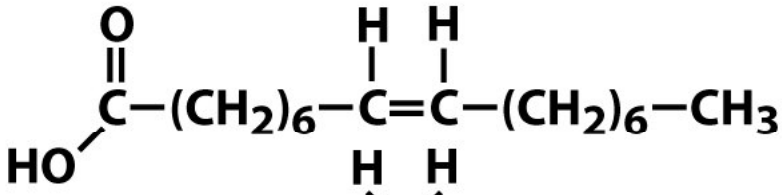
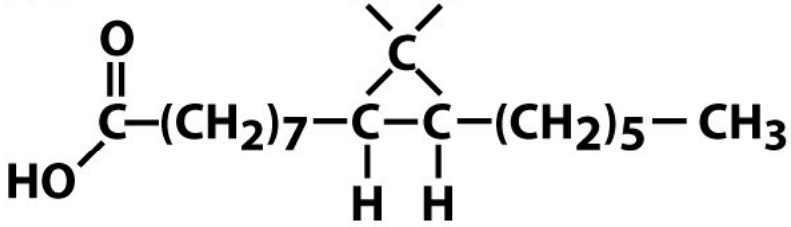
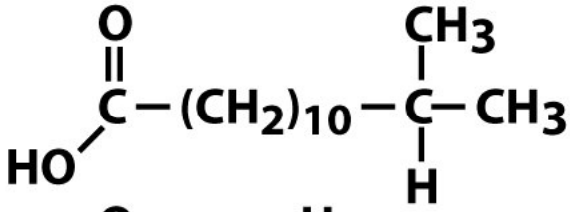
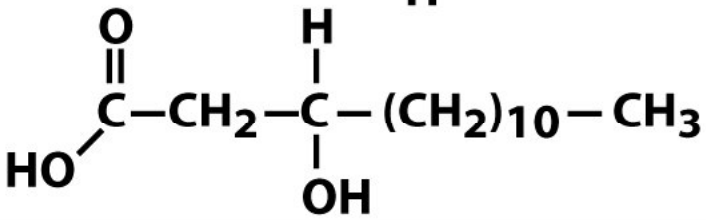


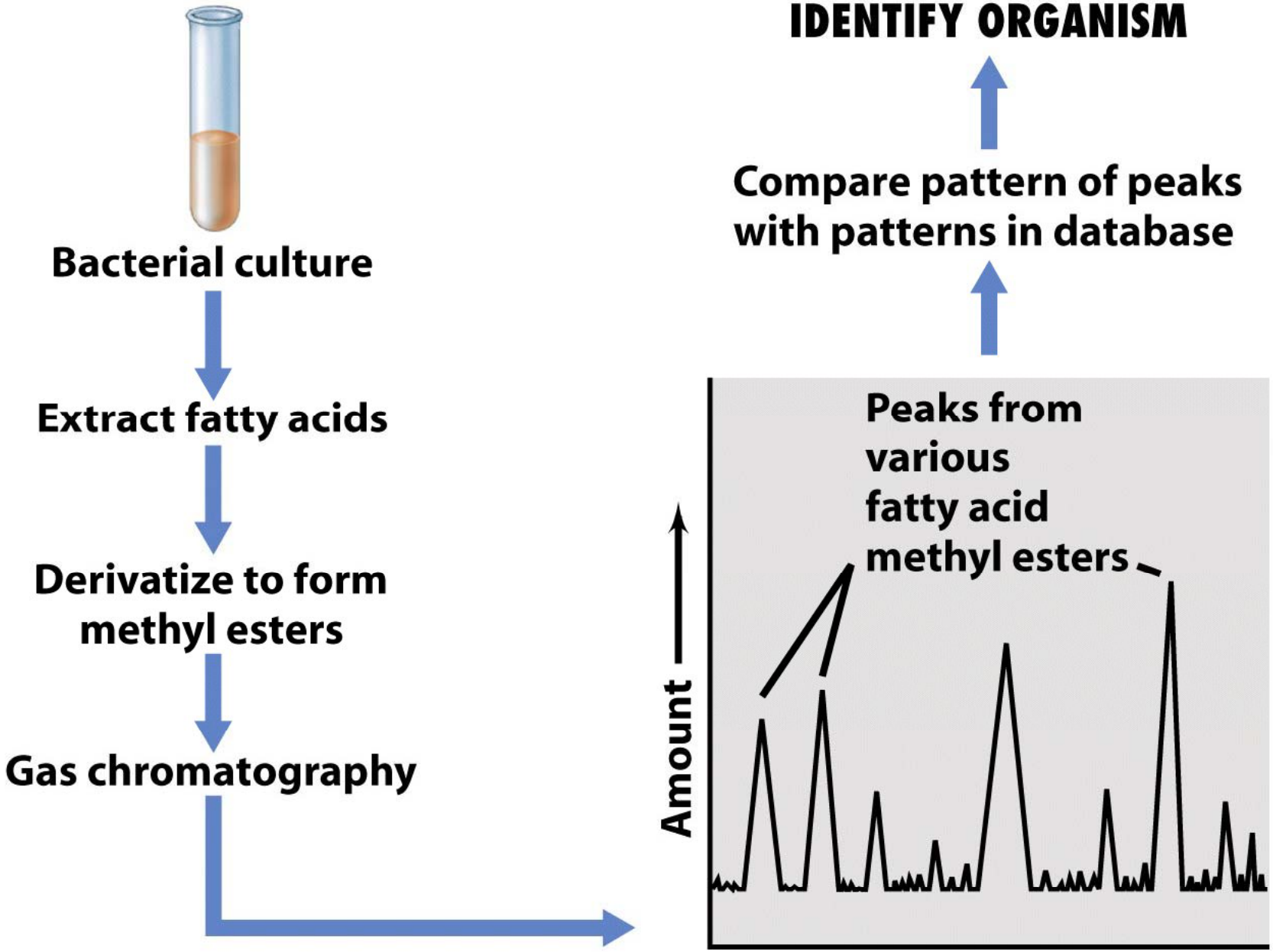
70% or greater; considered same species

FAME analysis

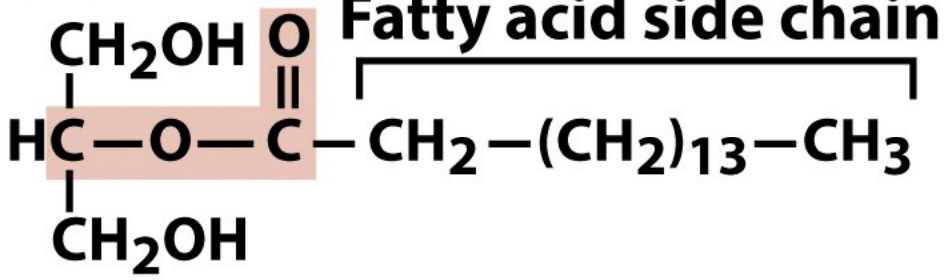


Classes of Fatty Acids in *Bacteria*

Class / Example	Structure of example
I. ***Saturated:*** tetradecanoic acid	
II. ***Unsaturated:*** *omega-7-cis* hexadecanoic acid	
III. ***Cyclopropane:*** *cis 7, 8* methylene hexadecanoic acid	
IV. ***Branched:*** 13-methyltetradecanoic acid	
V. ***Hydroxy:*** 3-hydroxytetradecanoic acid	

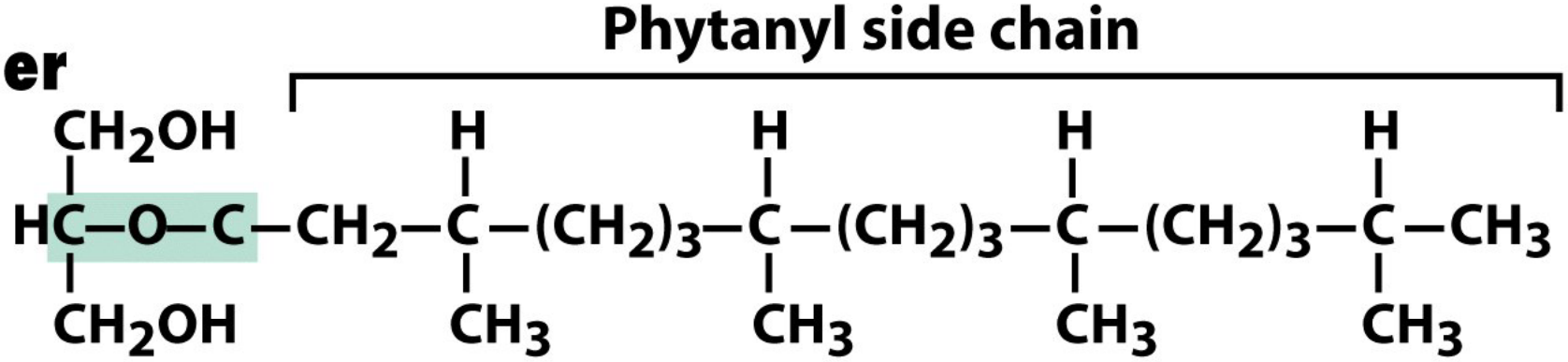

IDENTIFY ORGANISM
Compare pattern of peaks with patterns in database
Peaks from various fatty acid methyl esters
Amount
Bacterial culture
Extract fatty acids
Derivatize to form methyl esters
Gas chromatography

Ester



Bacteria, Eukarya

Ether



Archaea

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

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

Table 17.2 Taxonomic hierarchy of classification.

Taxon rank	A long-studied taxon	A less-studied taxon	An uncultivated environmental sample
Domain	**Bacteria**	**Archaea**	**Bacteria**
Division	**Actinobacteria**	**Euryarchaeota**	**Proteobacteria**
(phylum)	Filamentous gram-positive	Methanogens and halophiles	Purple bacteria and relatives; gram-negative
Class	**Actinobacteria**	**Methanococci**	**Alpha Proteobacteria**
	High GC gram-positive	Methanogens	Gram-negative bacteria
Subclass	**Actinobacteridae**		
Order	**Actinomycetales**	**Methanococcales**	**Rickettsiales**
	Filamentous; acid-fast stain	Methanogenic cocci	Includes intracellular bacteria
Suborder	**Streptomycineae**		
Family	**Streptomycetaceae**	**Methanocaldococcaceae**	**SAR11 cluster**
	Filamentous; hyphae produce spores	Thermophilic methanogens	Nonculturable planktonic marine bacteria
Genus	***Streptomyces***	***Methanocaldococcus***	***Pelagibacter***
Species	***S. coelicolor***	***M. jannaschii***	***P. ubique***
(date first described)	**(1908)**	**(1984)**	**(2002)**

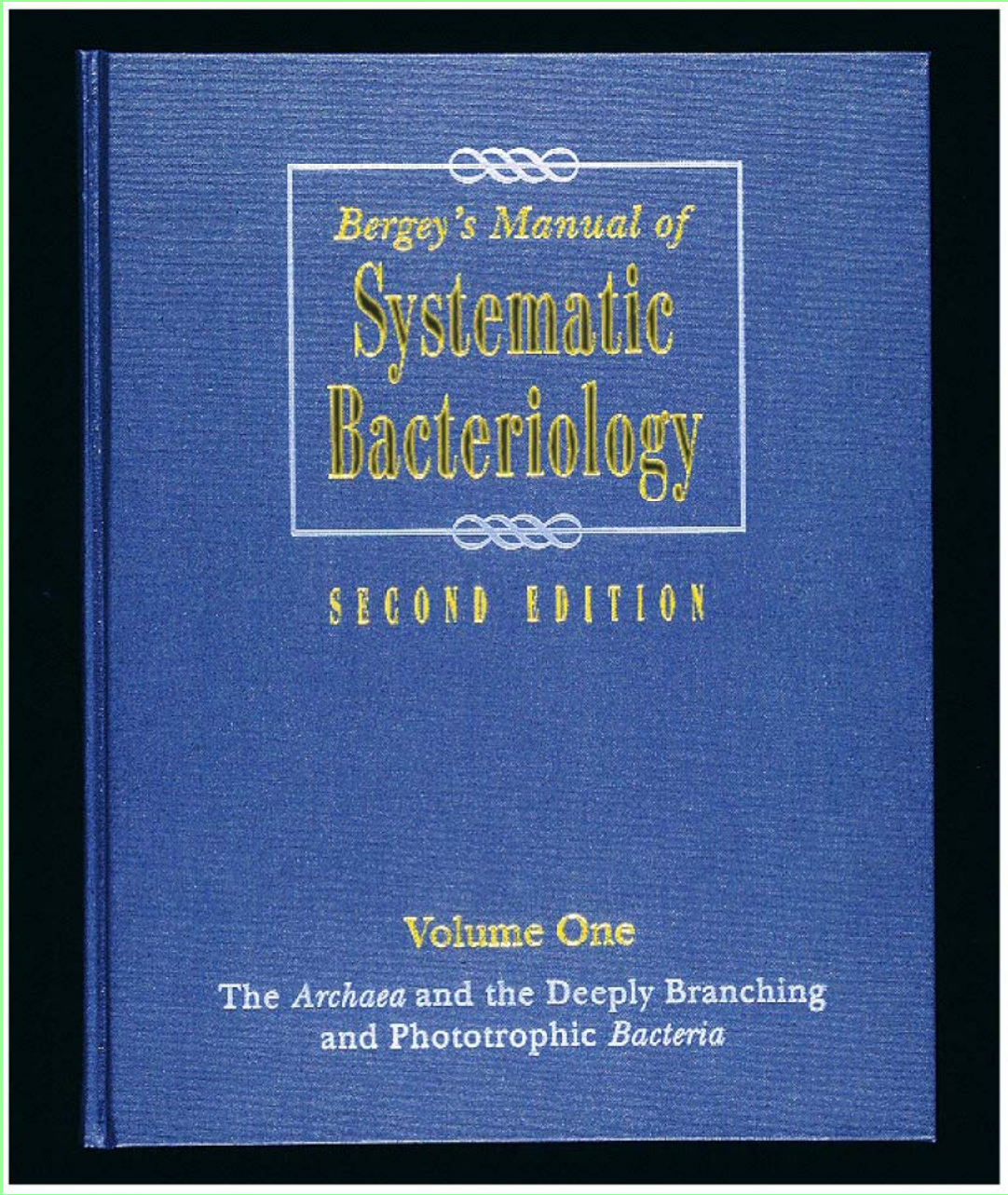
Table 17.2 Microbiology: An Evolving Science

Table 11.6 Taxonomic ranks and numbers of known prokaryotic species[a]

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

[a] 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.

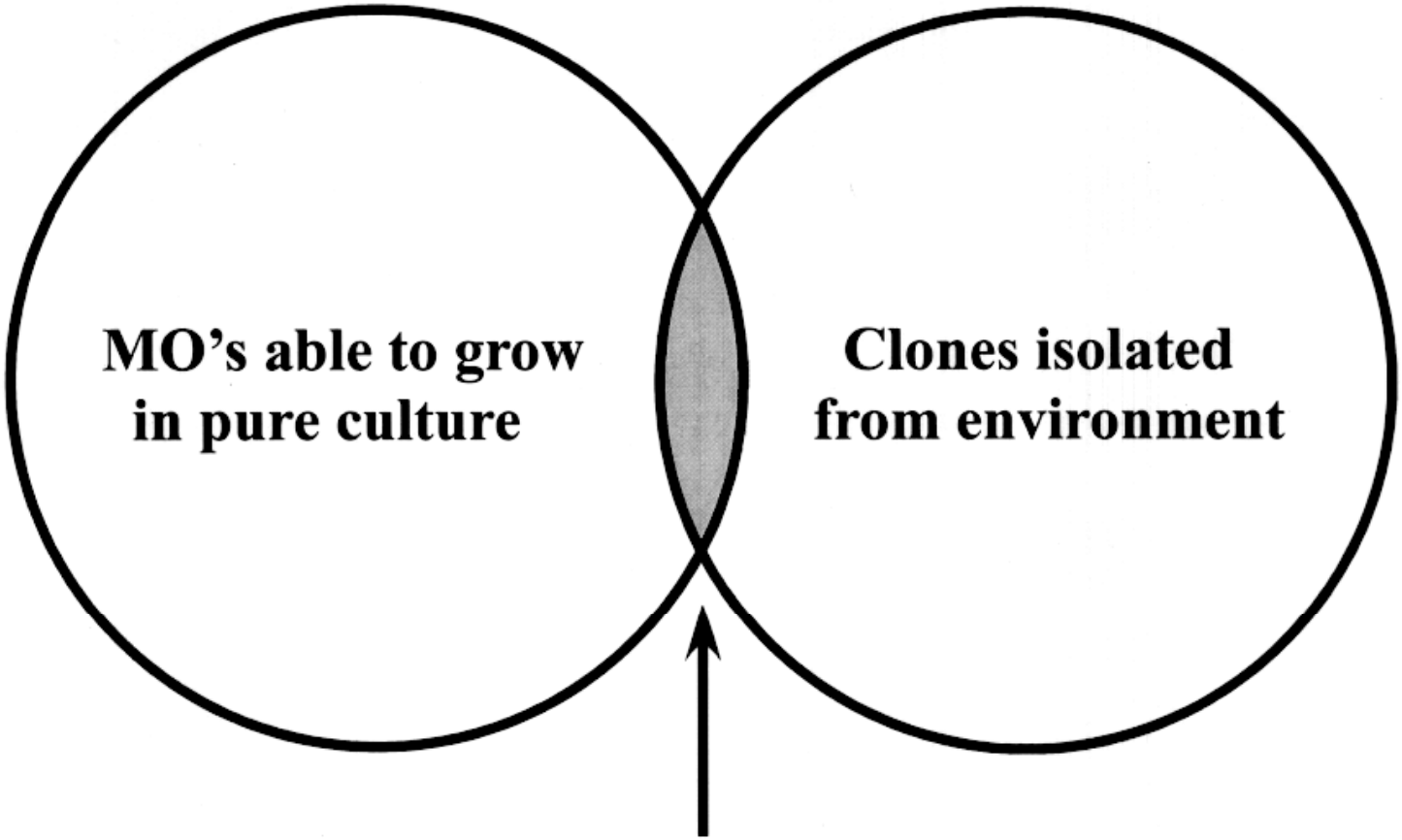

Bergey's Manual of
Systematic Bacteriology
SECOND EDITION
Volume One
The Archaea and the Deeply Branching and Phototrophic Bacteria

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 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.



>1% Crossover between these groups

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.

Stanier *et al.*, 1976:

... 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.*

Regarding Molecular Phylogeny

The Root of the Problem:

Unlike zoology and botany, microbiology developed without the knowledge of phylogenetic relationships among the organisms studied.

Molecular Phylogeny

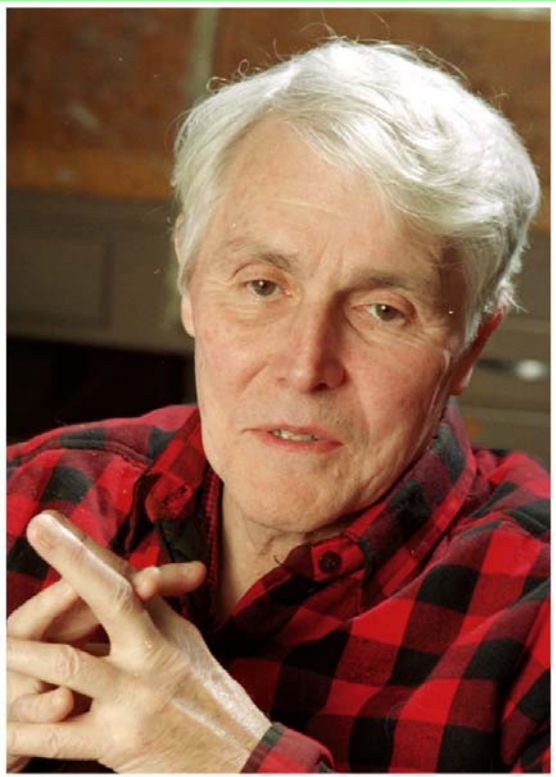


Figure 17.25 (inset) Microbiology: An Evolving Science
Courtesy of Carl Woese

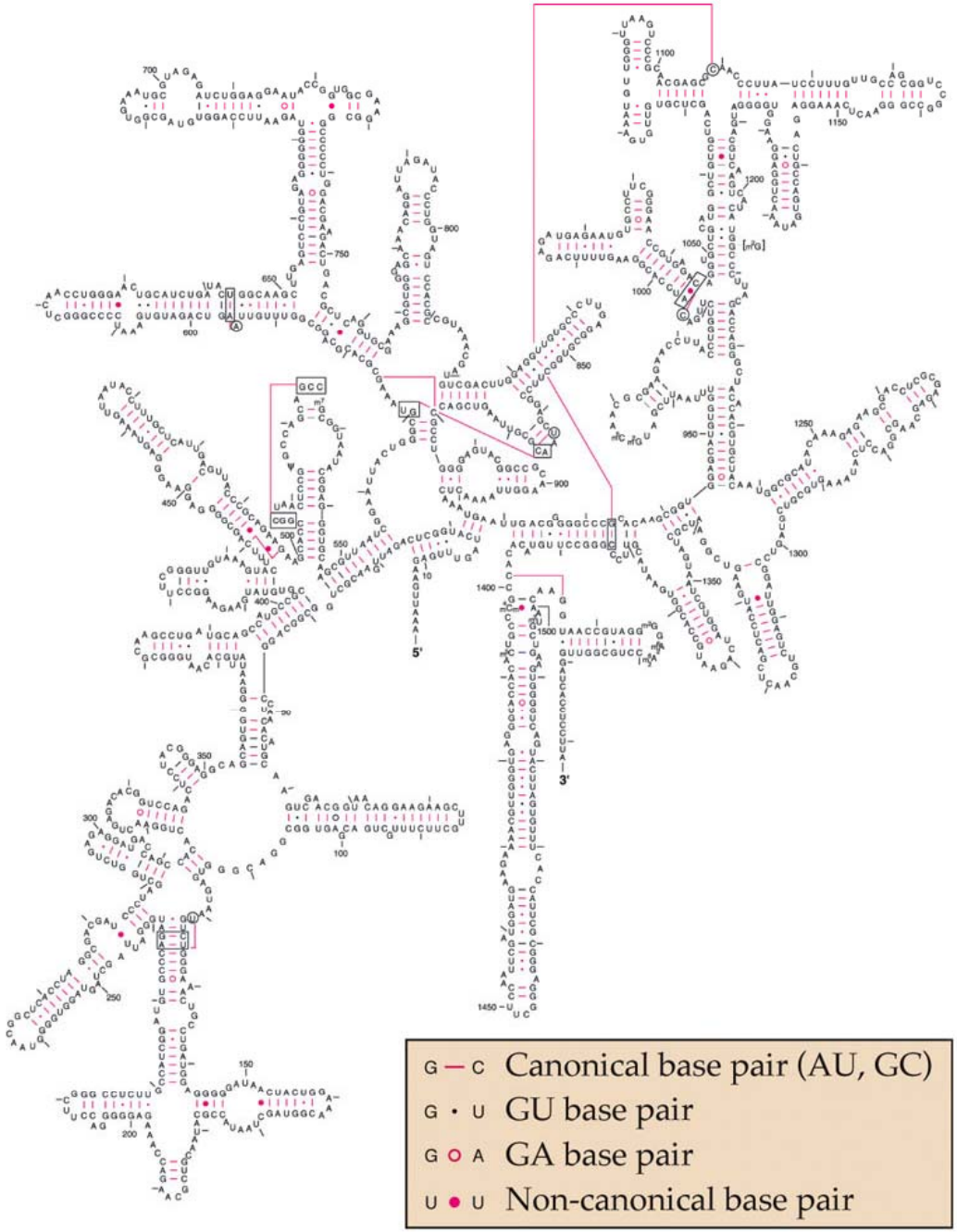
Woese (1977): Applied **rRNA** concept to redefine microbial systematics (microbial genealogy).

Pace (1984): Applied rRNA concept to microbial ecology (census without culturing).

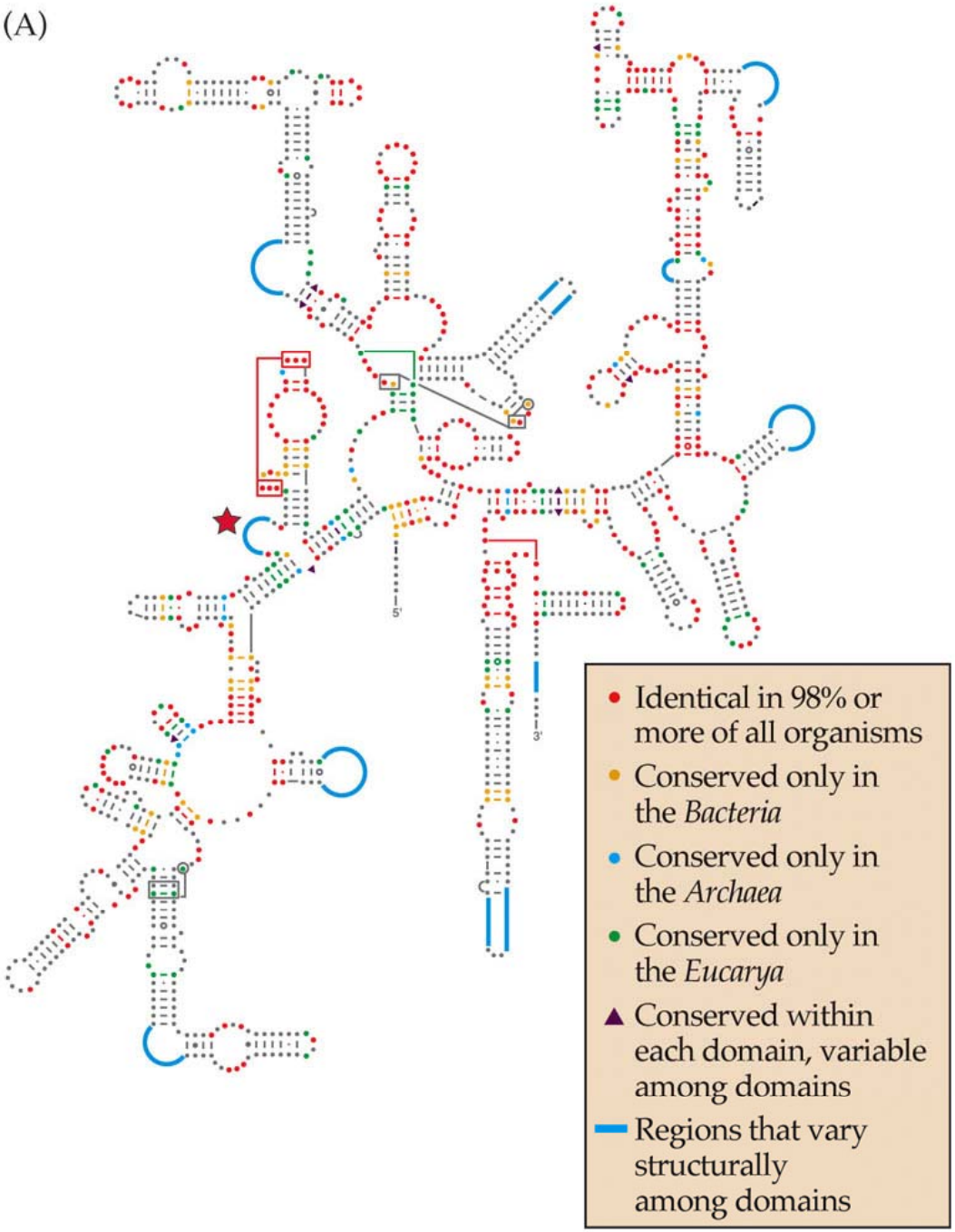
Figure 17.23a Microbiology: An Evolving Science
Courtesy of Norman R. Pace

Why ribosomal RNAs?

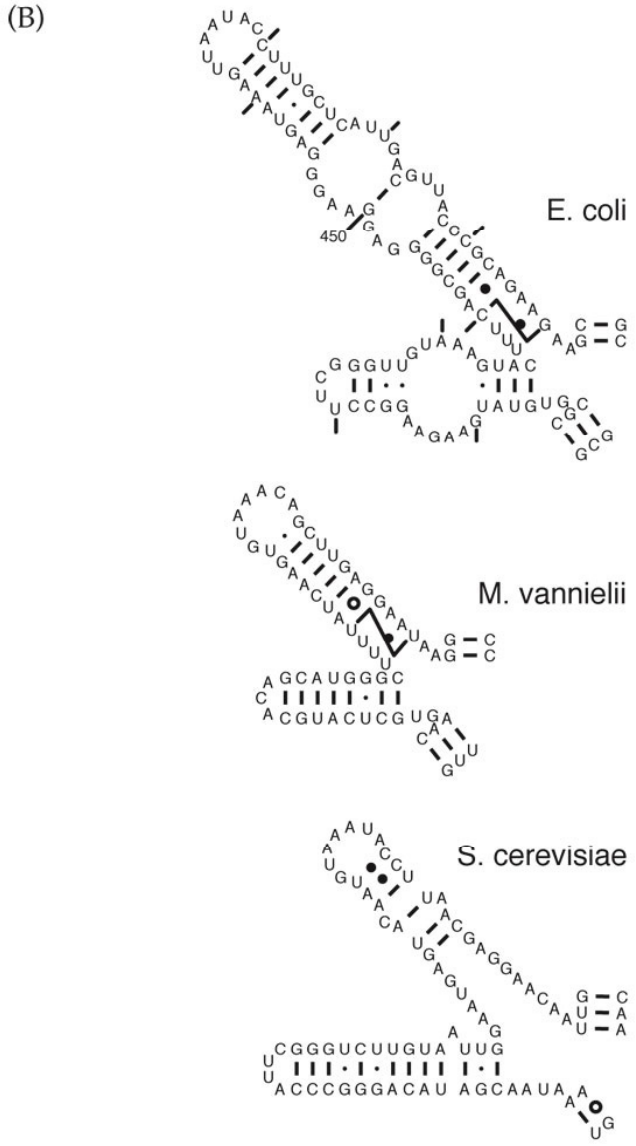
- Found among all living organisms (for 3.8 of the last 4.5 billion years). Integral part of protein synthesis machinery.
- Cell component analyses provide culture-independent means of investigating questions in microbial ecology (lack of morphology).
- rRNAs offer a type of sequence information that makes them excellent descriptors of an organism's evolutionary history.
- No detectable horizontal gene transfer, especially important for the bacteria and archaea.
- Large and growing database; RDP contains ~1.3×10^6 SSU rRNAs.

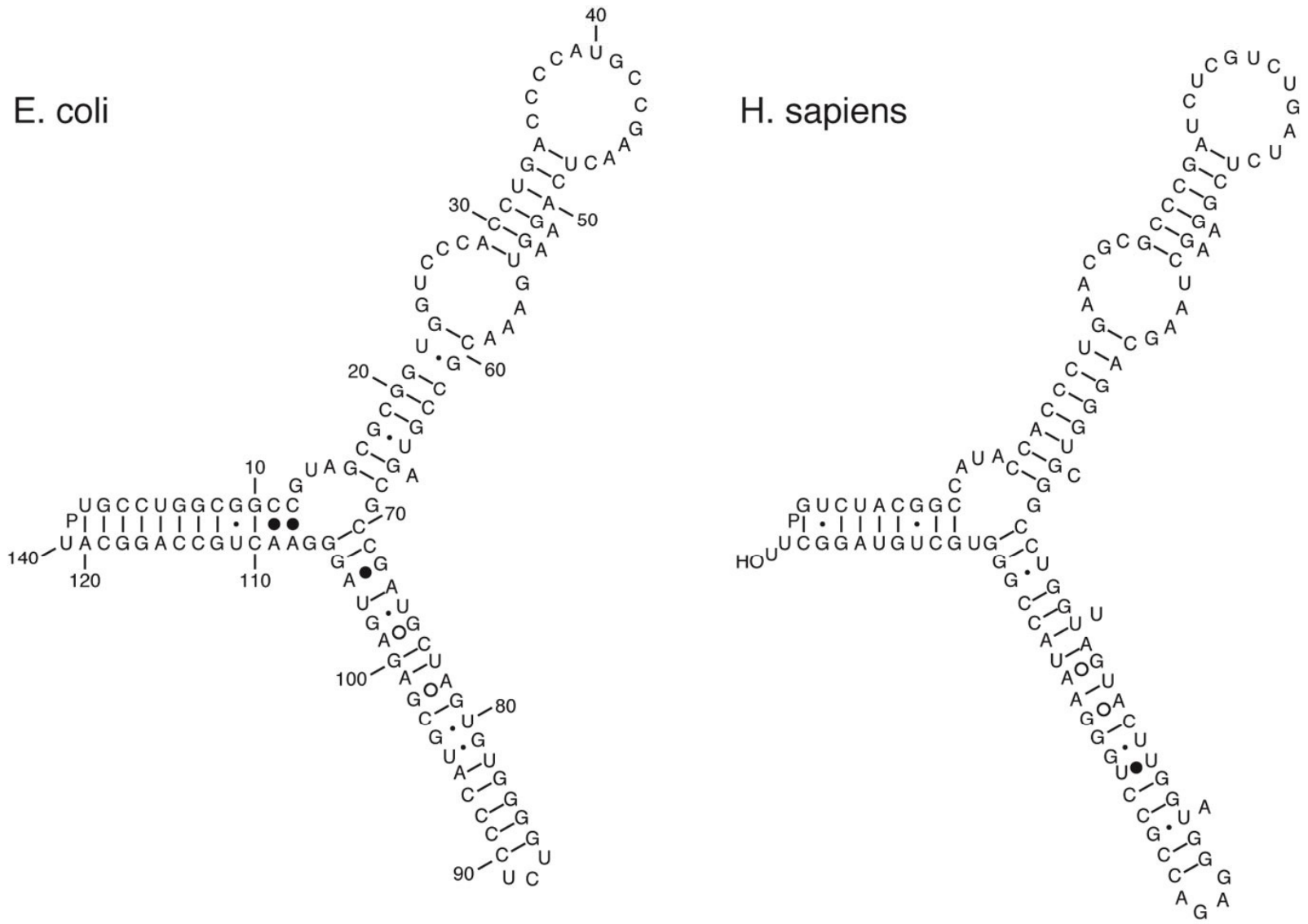

G — C Canonical base pair (AU, GC)
G • U GU base pair
G o A GA base pair
U • U Non-canonical base pair

(A)


5'
3'
Identical in 98% or more of all organisms
Conserved only in the *Bacteria*
Conserved only in the *Archaea*
Conserved only in the *Eucarya*
Conserved within each domain, variable among domains
Regions that vary structurally among domains

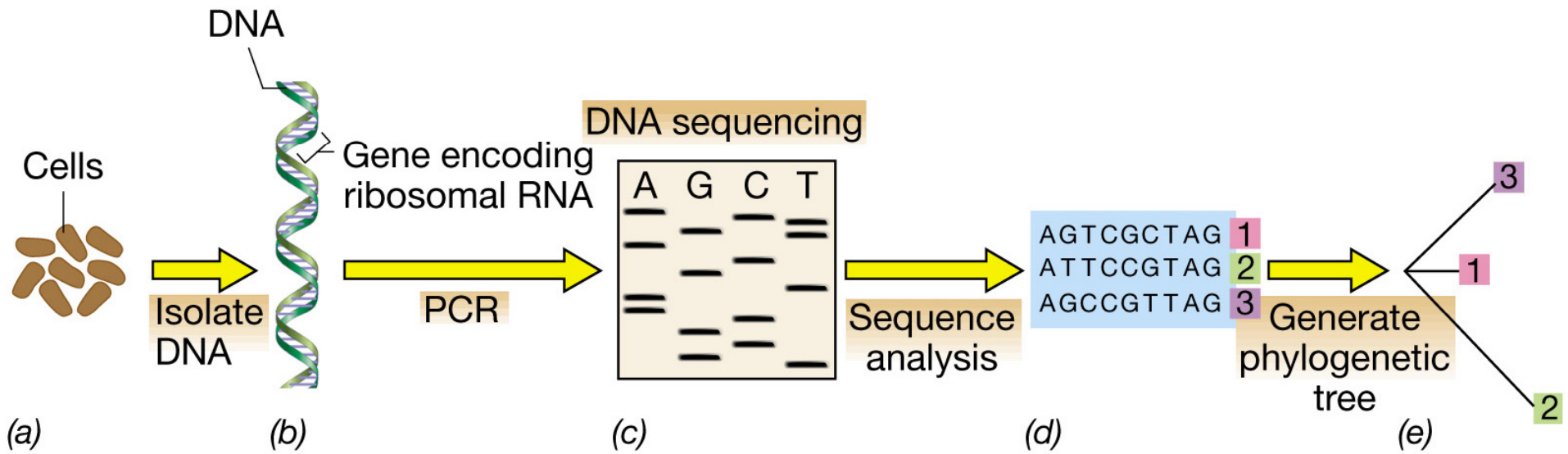
Secondary Structures of SSU rRNA show homology





Secondary Structures of rRNAs show homology

Molecular Strategy Flow Chart



Note: Independent of pure culture isolation!

Estimating evolutionary distance E_D to map on phylogenetic tree

Organism	Sequence
A	CGUAGACCUGAC
B	CCUAGAGCUGGC
C	CCAAGACGUGGC
D	GCUAGAUGUGCC

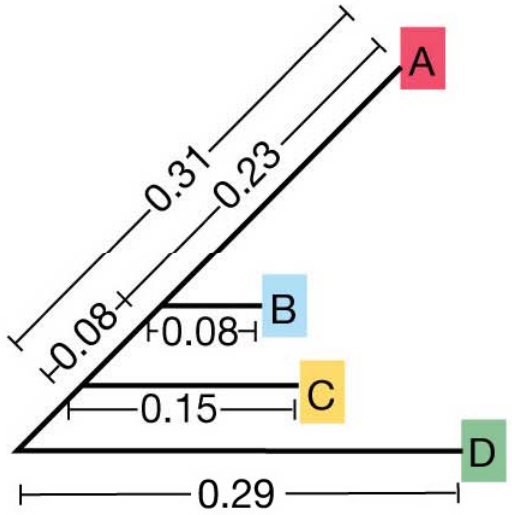
Analysis

For A ⟶ B, three differences occur out of a total of twelve; thus $\frac{3}{12} = 0.25$

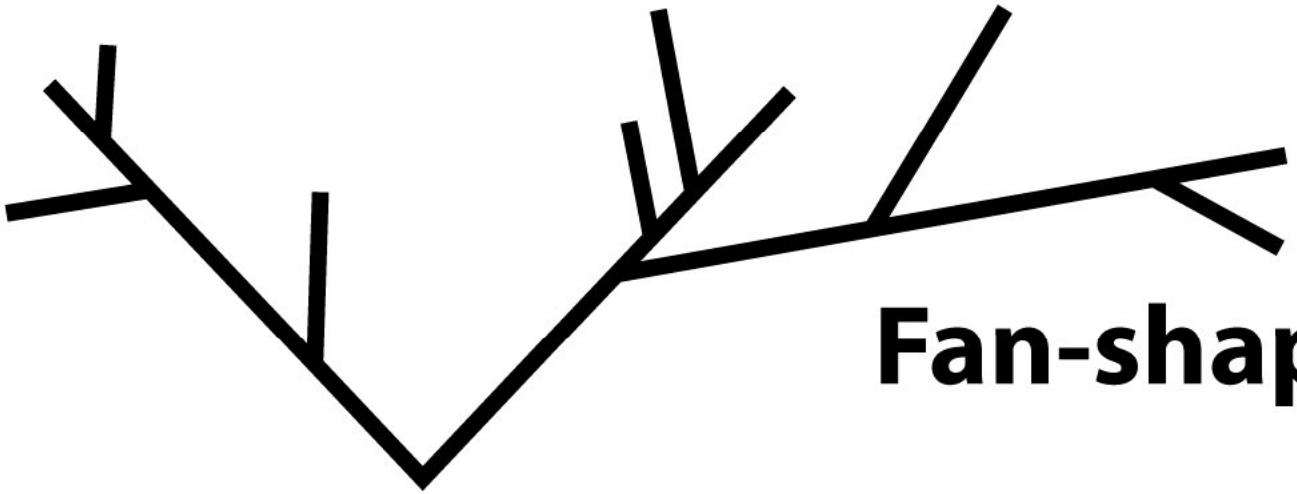
(a) **Sequence alignment and analysis**

Evolutionary distance			Corrected evolutionary distance
E_D	A ⟶ B	0.25	0.30
E_D	A ⟶ C	0.33	0.44
E_D	A ⟶ D	0.42	0.61
E_D	B ⟶ C	0.25	0.30
E_D	B ⟶ D	0.33	0.44
E_D	C ⟶ D	0.33	0.44

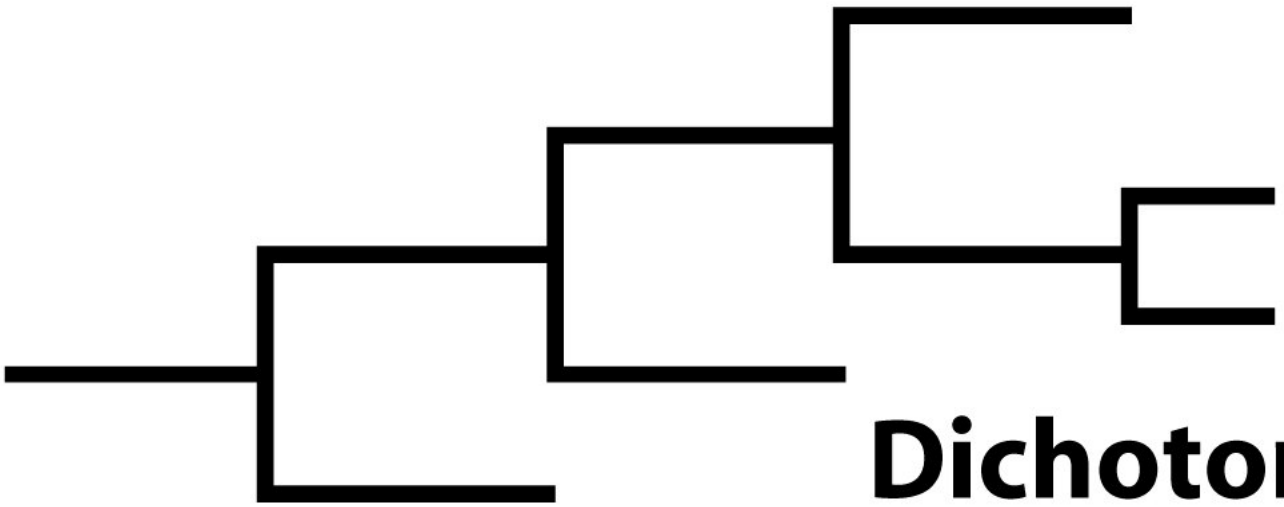
(b) **Calculation of evolutionary distance**



(c) **Phylogenetic tree**

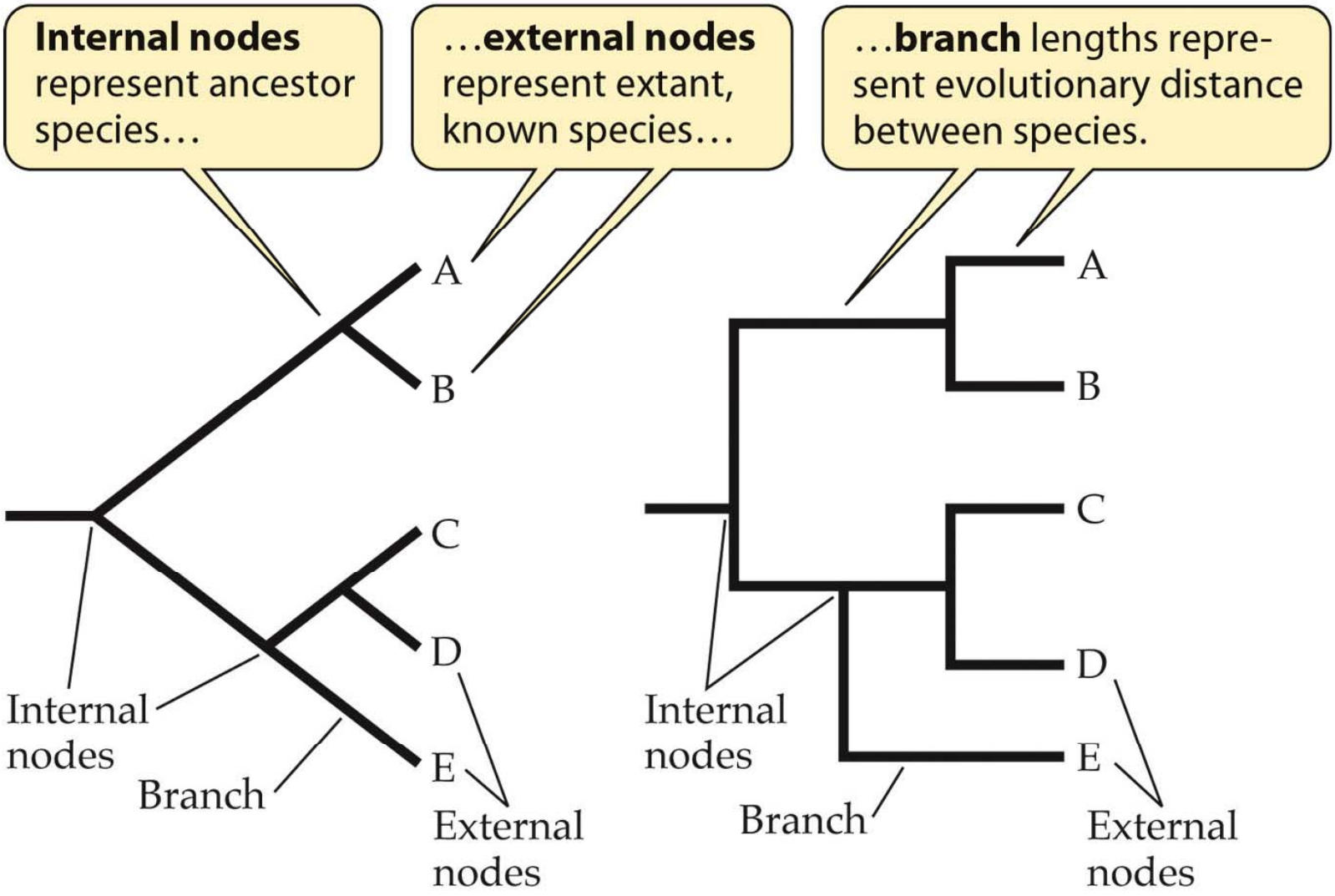


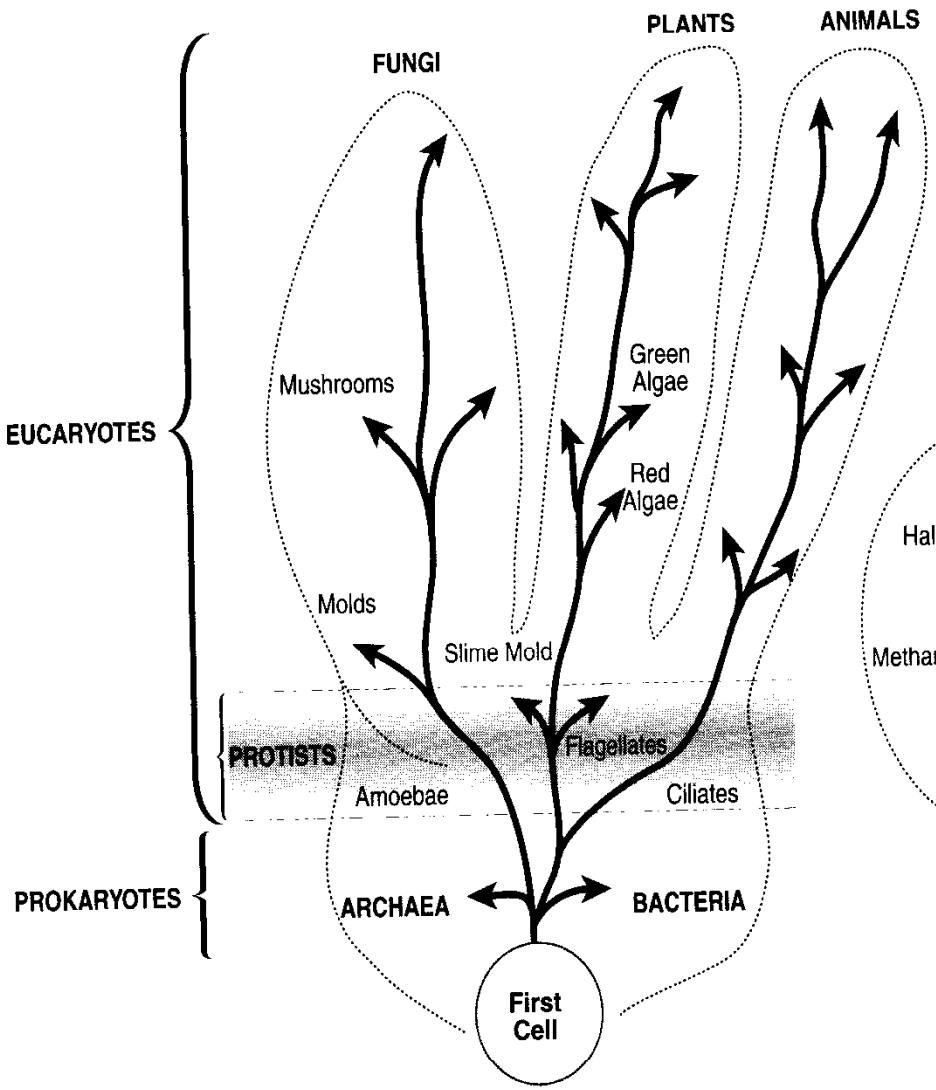
Fan-shaped



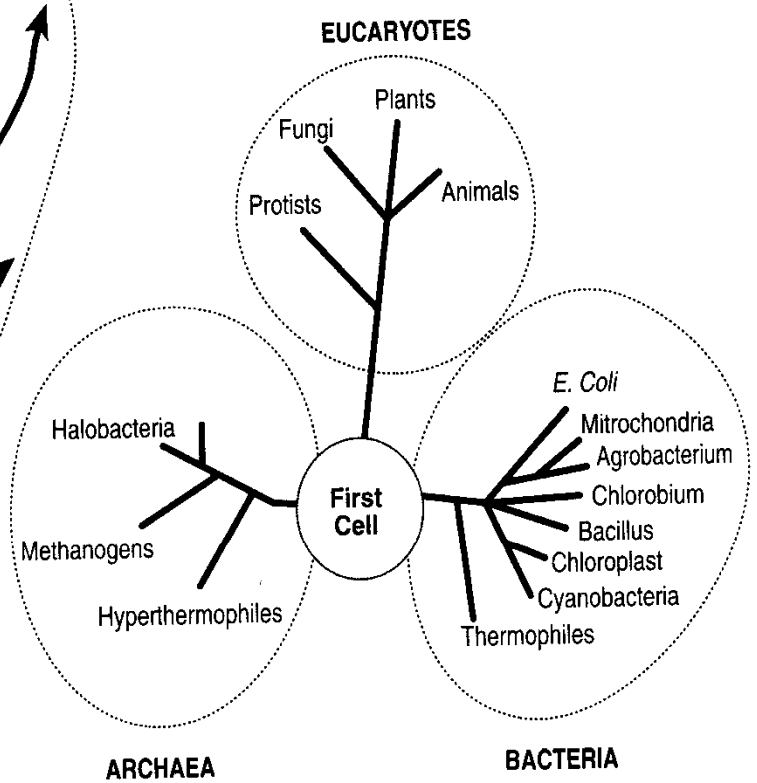
Dichotomous

Tree topologies


Internal nodes represent ancestor species…
…**external nodes** represent extant, known species…
…**branch** lengths represent evolutionary distance between species.
A
B
C
D
E
Internal nodes
Branch
External nodes
A
B
C
D
E
Internal nodes
Branch
External nodes



"CLASSICAL" PHYLOGENY



MOLECULAR PHYLOGENY

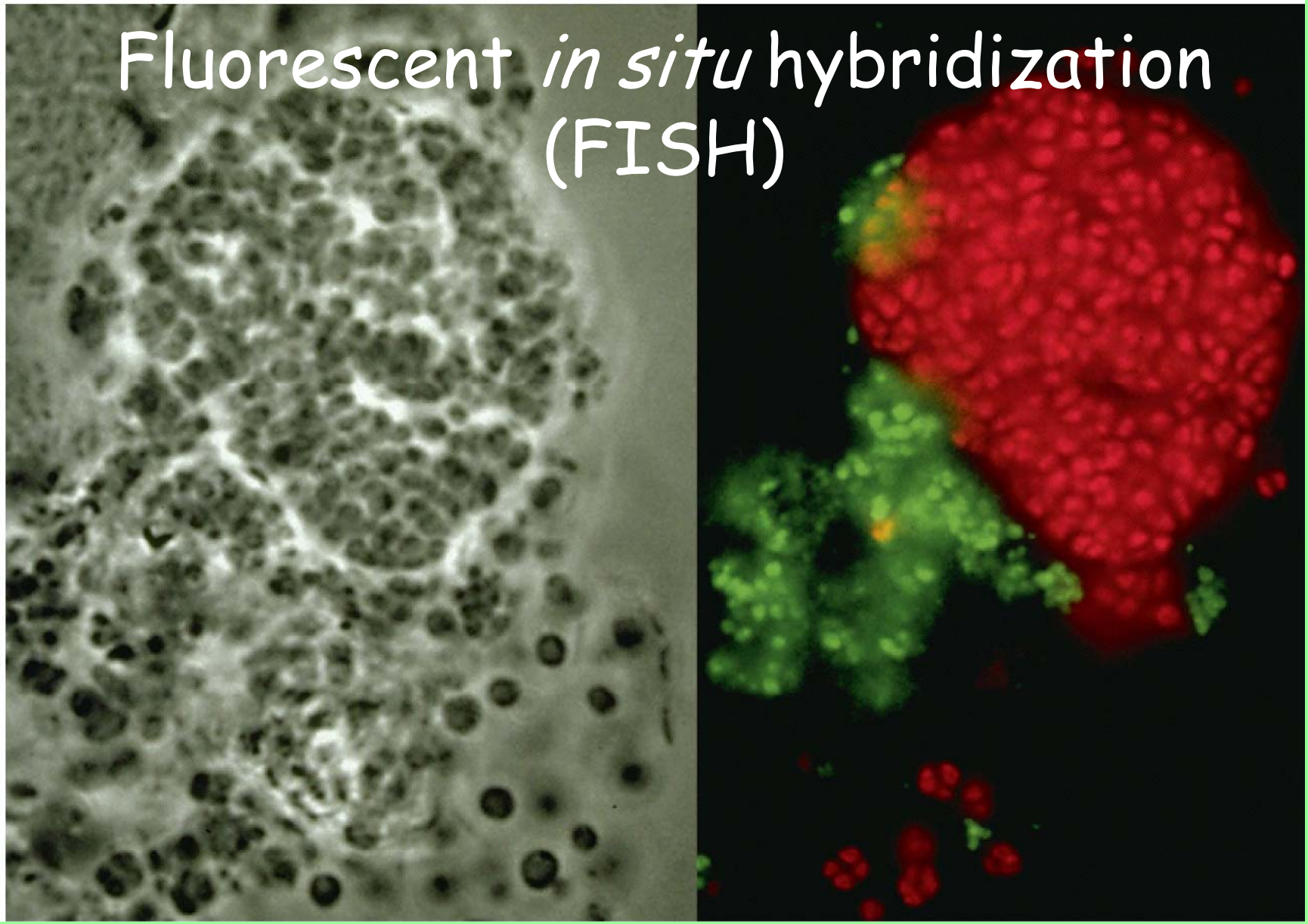
Table 11.1 **Signature sequences from 16S or 18S rRNA defining the three domains of life**

		Occurrence among[c]		
Oligonucleotide signatures[a]	**Approximate position[b]**	***Archaea***	***Bacteria***	***Eukarya***
CACYYG	315	0	>95	0
AAACUCAAA	910	3	100	0
AAACUUAAAG	910	100	0	100
YUYAAUUG	960	100	<1	100
CAACCYYCR	1110	0	>95	0
UCCCUG	1380	>95	0	100
UACACACCG	1400	0	>99	100
CACACACCG	1400	100	0	0

[a] Y, any pyrimidine; R, any purine.
[b] Refer to Figure 11.11*c* for numbering scheme of 16S rRNA.
[c] 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

Fluorescent *in situ* hybridization (FISH)



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)

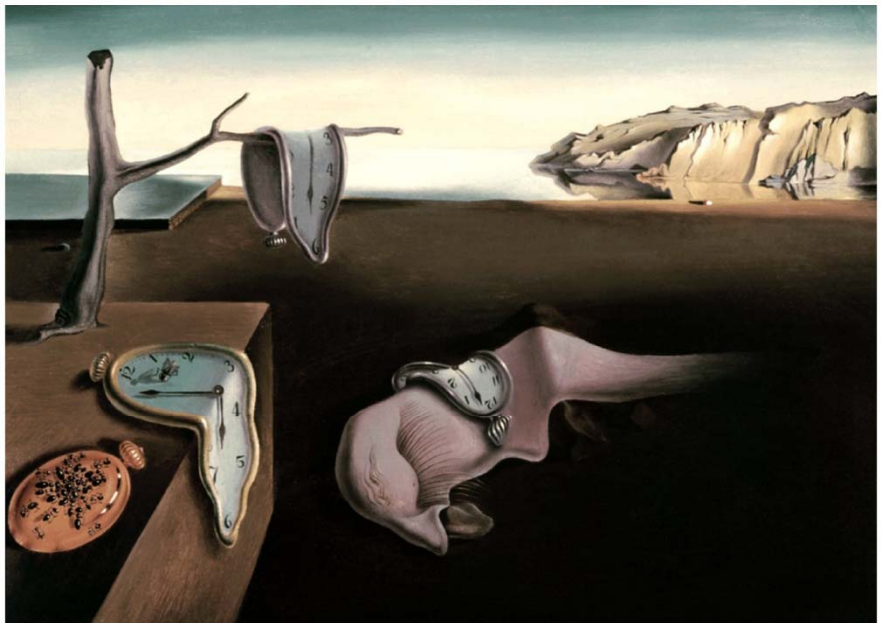
Inferring evolutionary relationships from a phylogenetic tree

-Key word is inference (not always correct!)

-Some lineages accumulate mutations faster than others – generation times and selective pressures differ.

So, molecular clocks are distorted ("soft watches").

-For this reason, mutation frequency cannot be calibrated to units of time. Tree can be calibrated to fossil record or geological evidence.



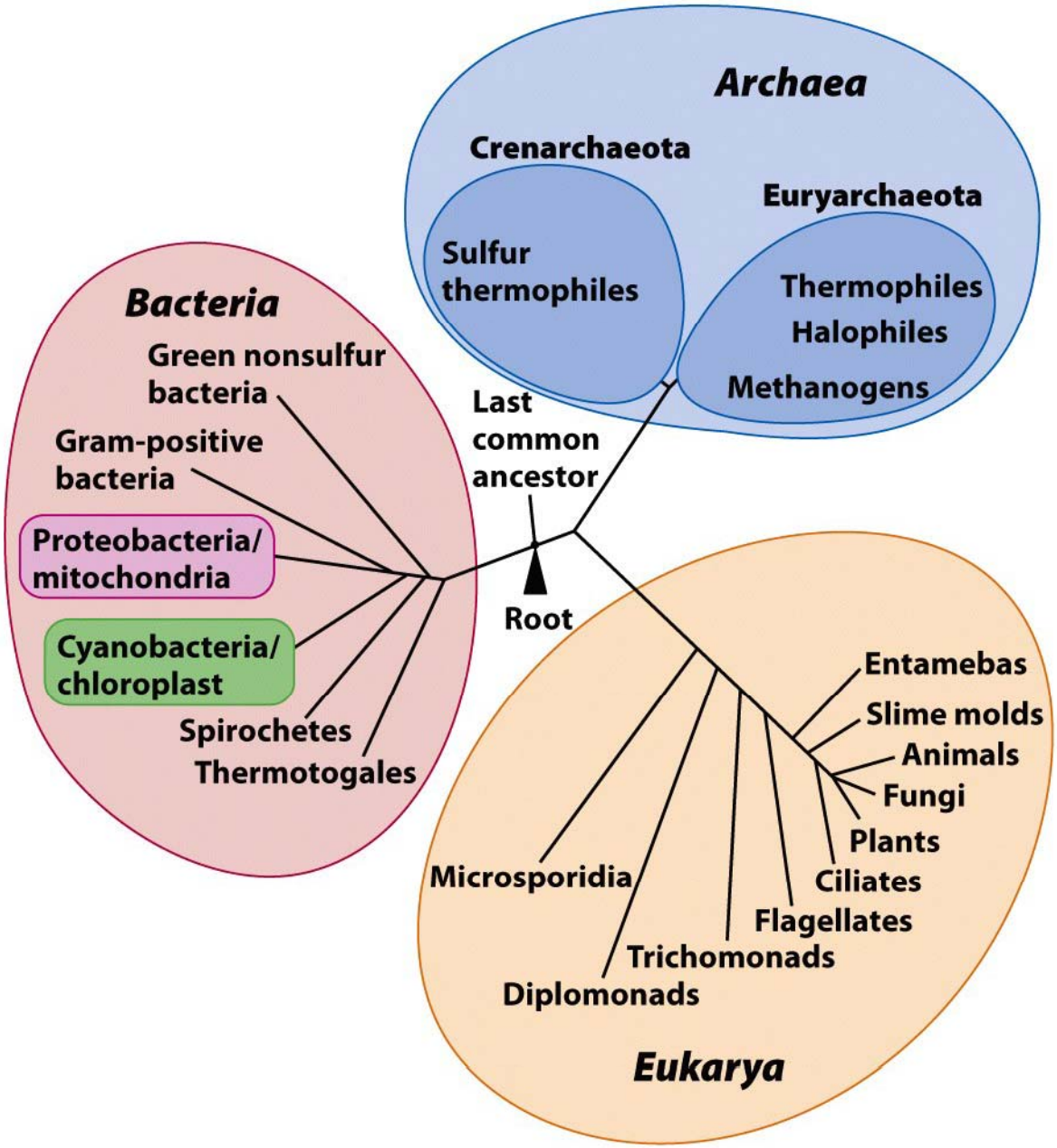


Figure 17.25 Microbiology: An Evolving Science

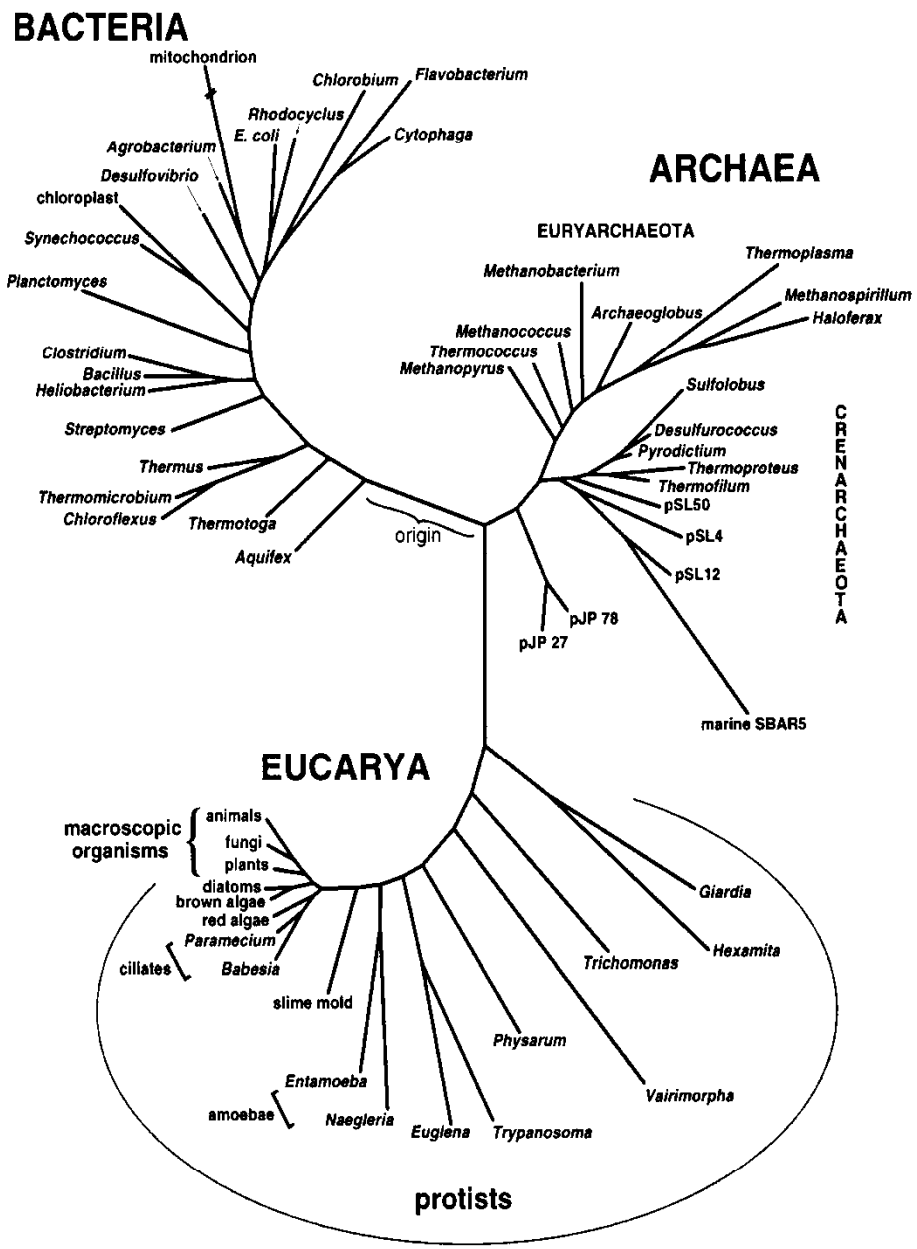


Figure 1. Diagrammatic "Universal" phylogenetic tree of life, based on small-subunit ribosomal RNA sequences. Based on analyses of Barns et al. (1996b), Olsen et al. (1994), and Sogin (1994).

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

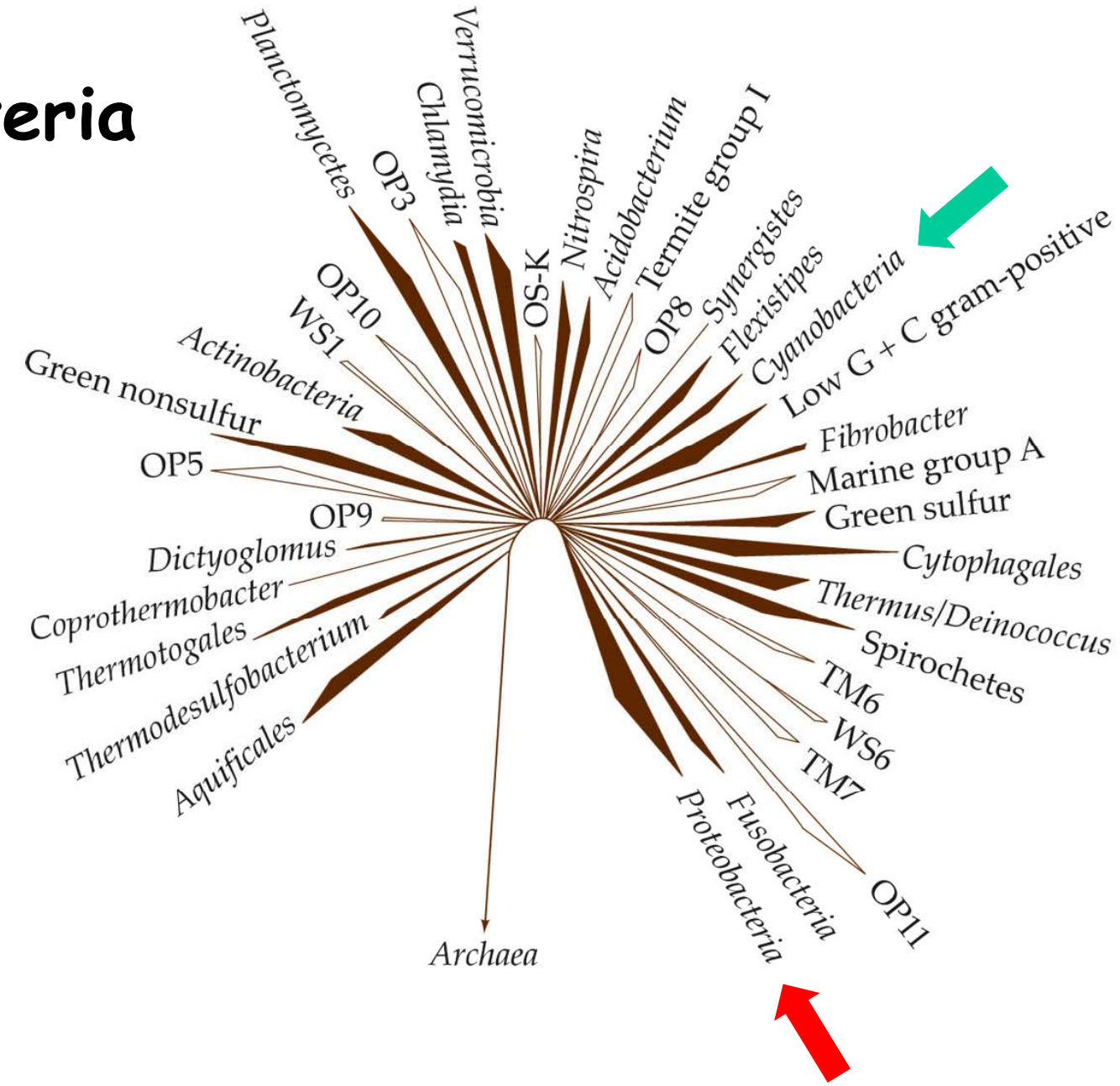
Single origin for all life on Earth...

- 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.

Bacteria


Planctomycetes
OP3
Chlamydia
Verrucomicrobia
OS-K
Nitrospira
Acidobacterium
Termite group I
OP8
Synergistes
Flexistipes
Cyanobacteria
Low G + C gram-positive
Fibrobacter
Marine group A
Green sulfur
Cytophagales
Thermus/Deinococcus
Spirochetes
TM6
WS6
TM7
OP11
Fusobacteria
Proteobacteria
Archaea
OP10
WS1
Actinobacteria
Green nonsulfur
OP5
OP9
Dictyoglomus
Coprothermobacter
Thermotogales
Thermodesulfobacterium
Aquificales

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

Table 17.3 Three domains of life.

Characteristic	Traits of living organisms		
	All cells on Earth resemble each other		
Chromosomal material	Double-stranded DNA		
RNA transcription	Common ancestral RNA polymerase		
Translation	Common ancestral rRNAs and elongation factors		
Protein	Common ancestral functional domains		
Cell structure	Aqueous cell compartment bounded by a membrane		
	Comparison of domains		
	Bacteria	**Archaea**	**Eukaryotes**
	Archaea resemble bacteria		
Cell volume	1–100 μm^3 (usually)		1–10^6 μm^3
DNA chromosome	Circular (usually)		Linear
DNA organization	Nucleoid		Nucleus with membrane
Gene organization	Multigene operons		Single genes
Metabolism	Denitrification, N_2 fixation, lithotrophy, respiration, and fermentation		Respiration and fermentation
Multicellularity	Simple		Simple or complex

Table 17.3 part 1 Microbiology: An Evolving Science

Table 17.3 Three domains of life.

Characteristic	Traits of living organisms		
		Archaea resemble eukaryotes	
Intron splicing	Introns are rare	Introns are common	
RNA polymerase	Bacterial	Eukaryotic form	
Transcription factors	Bacterial	Eukaryotic form	
Ribosome sensitivity to chloramphenicol, kanamycin, and streptomycin	Sensitive	Resistant	
Translation initiator	Formylmethionine	Methionine (except mitochondria use formylmethionine)	
Cell wall	Peptidoglycan	Pseudopeptidoglycan or other polymer; or protein S-layer	
	Bacteria resemble eukaryotes and differ from archaea		
Methanogenesis	No	Yes	No
Thermophilic growth	Up to 90°C	Up to 120°C	Up to 70°C
Photosynthesis	Many species; bacteriochlorophyll Proteorhodopsin derived from archea	Haloarchaea only; bacteriorhodopsin	Many species; chlorophyll (bacterial origin)
Chlorophyll light absorption	Red and blue	Green (central range of solar spectrum)	Red and blue (chloroplasts of bacterial origin)
Membrane lipids (major)	Ester-linked fatty acids	Ether-linked isoprenoid	Ester-linked fatty acids
Pathogens infecting animals or plants	Many pathogens	No pathogens	Many pathogens

Table 17.3 part 2 Microbiology: An Evolving Science

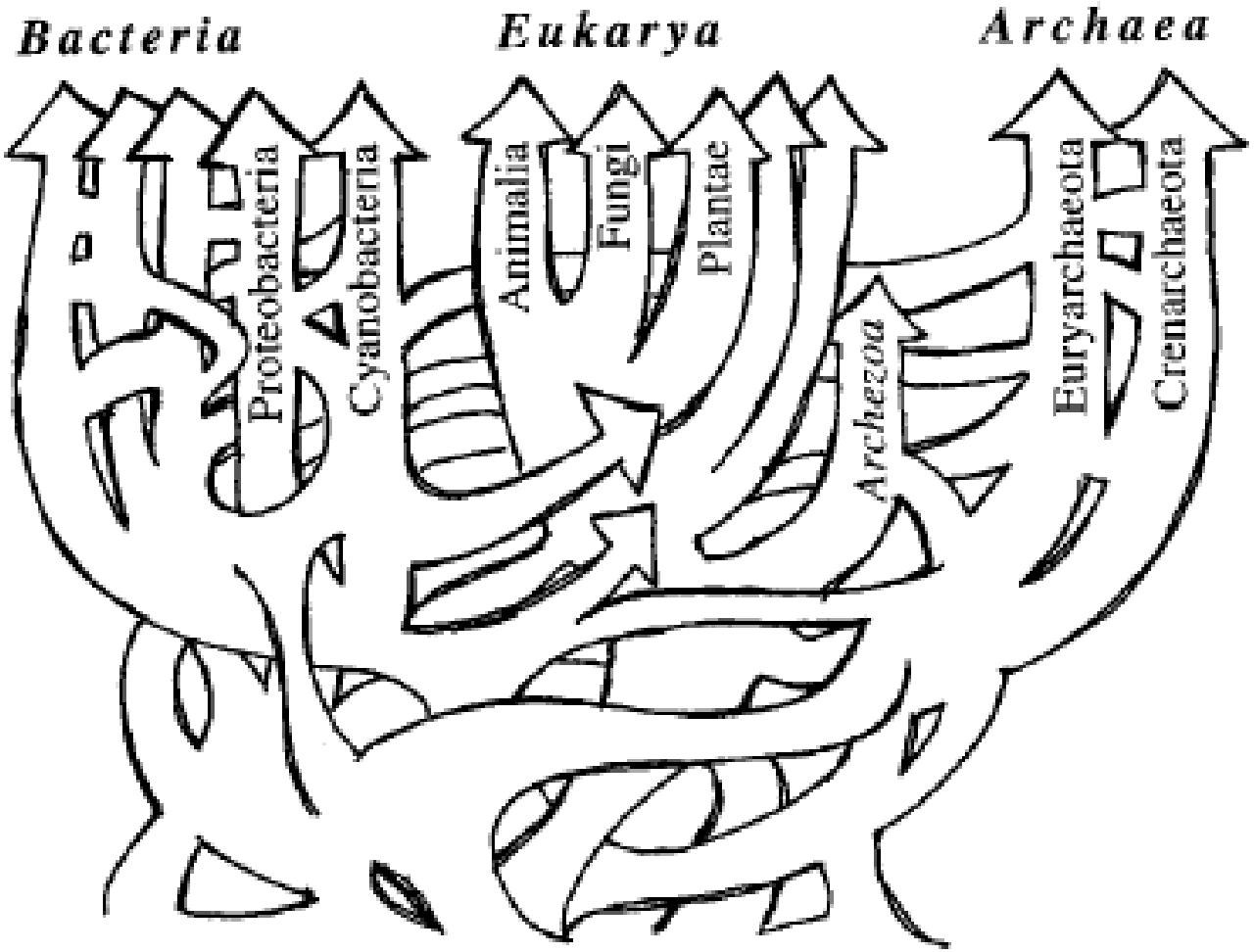
Horizontal gene transfer

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!

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



Doolittle's Universal Tree (1999)

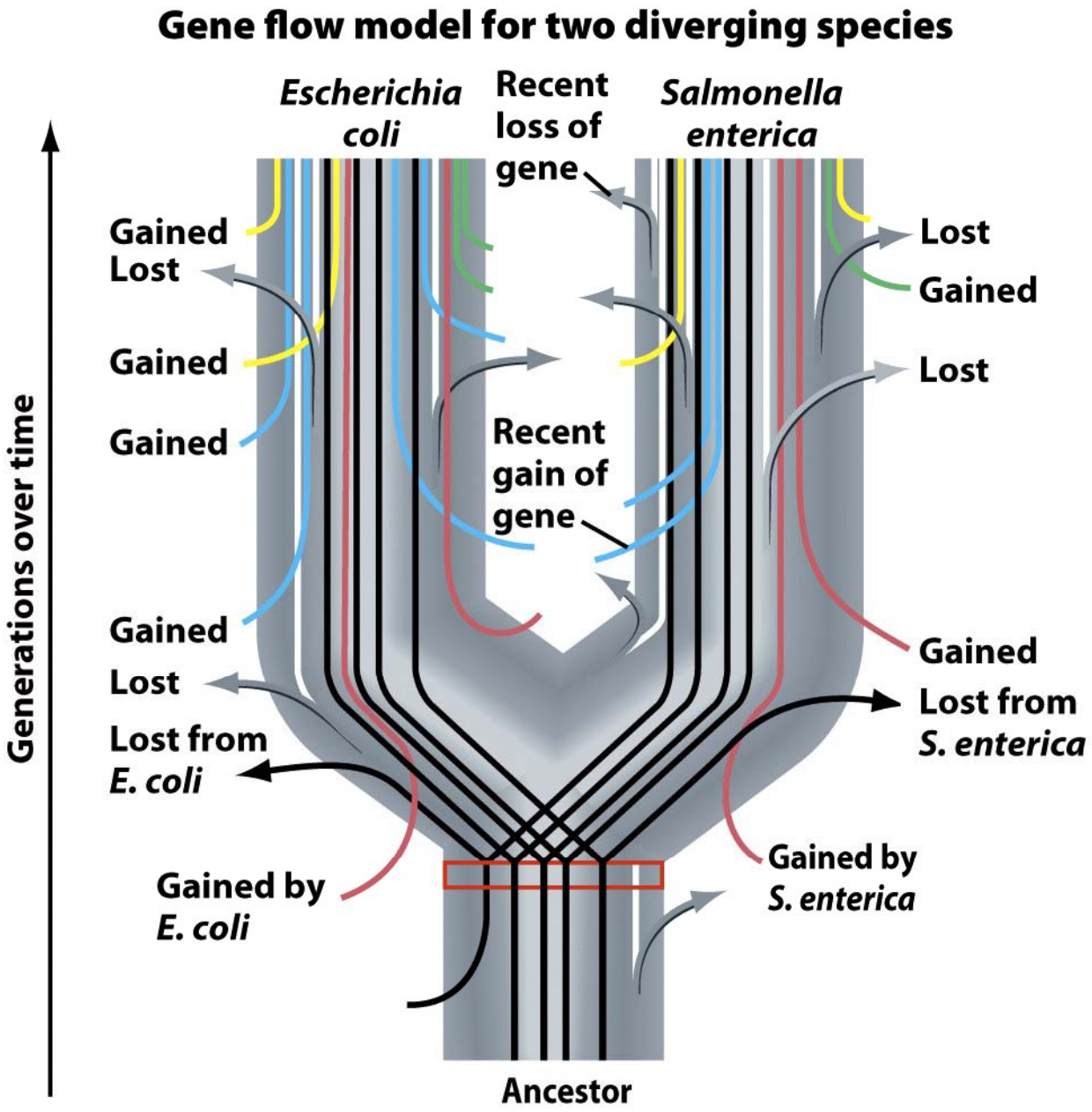
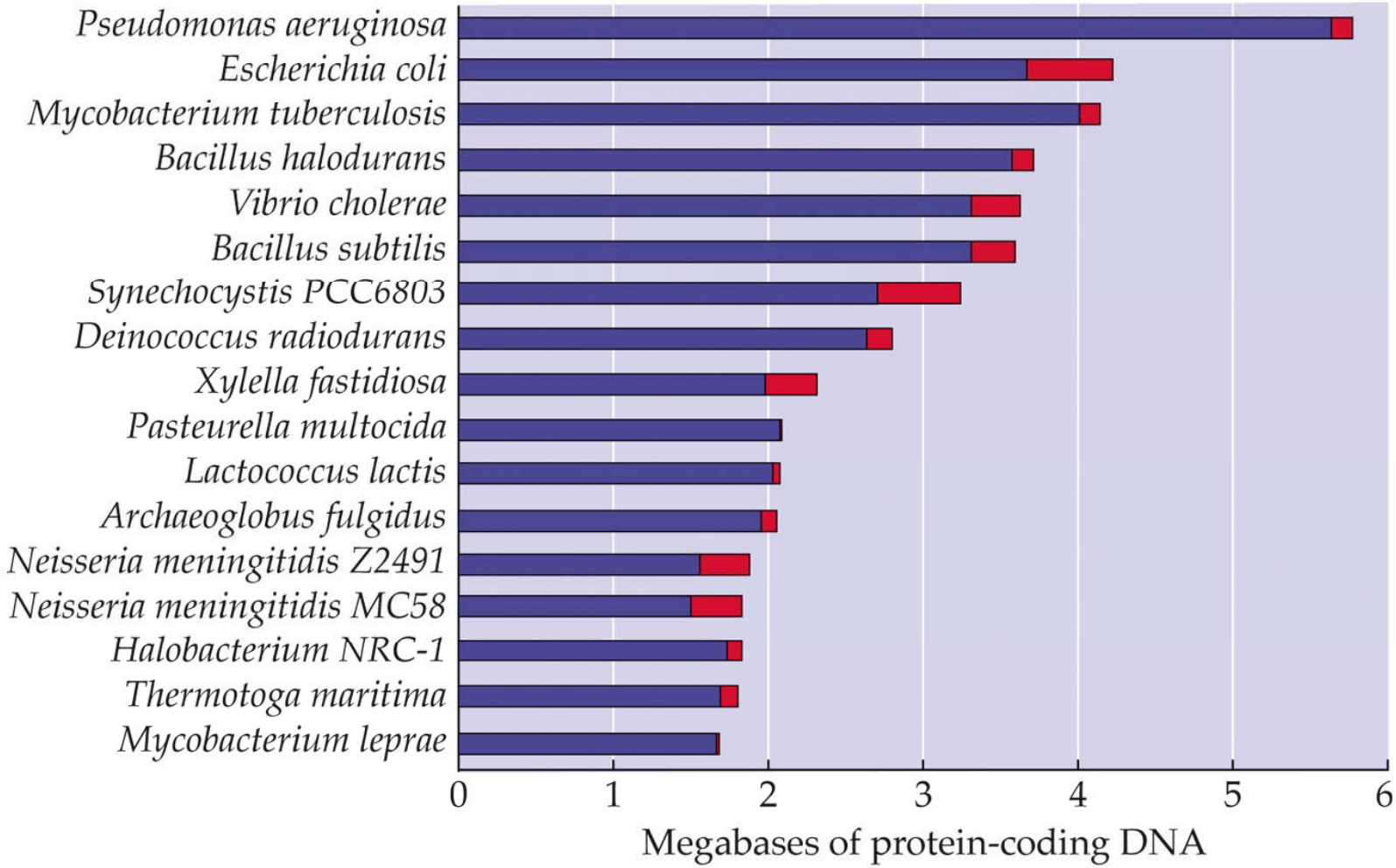
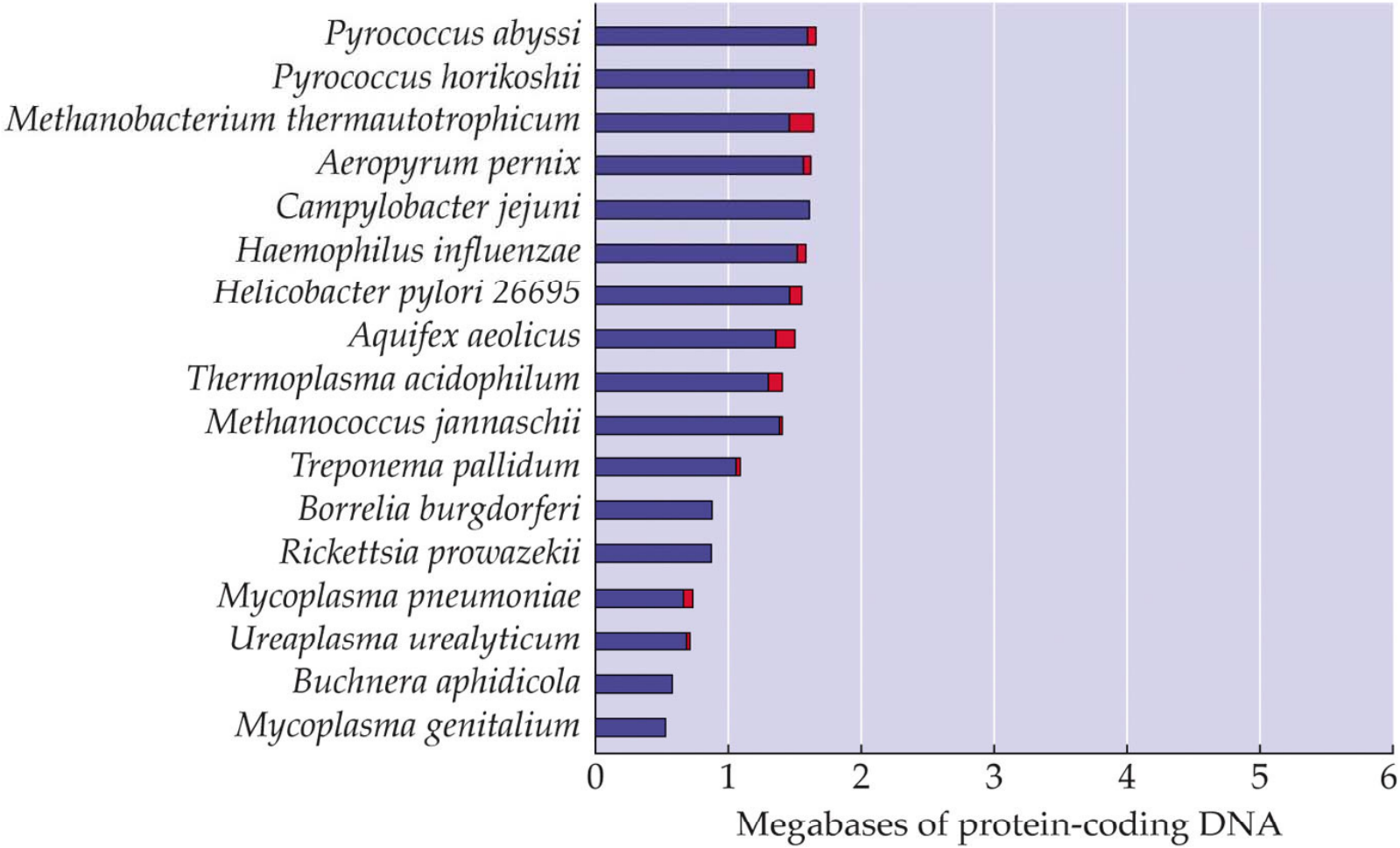


Figure 17.28 Microbiology: An Evolving Science

Relative importance of horizontal gene transfer



Relative importance of horizontal gene transfer



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.

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** (~5×10^6 bp genome), we are **complex** (~3×10^9 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.

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

TABLE 13.3 C values from eukaryotic organisms ranked by size

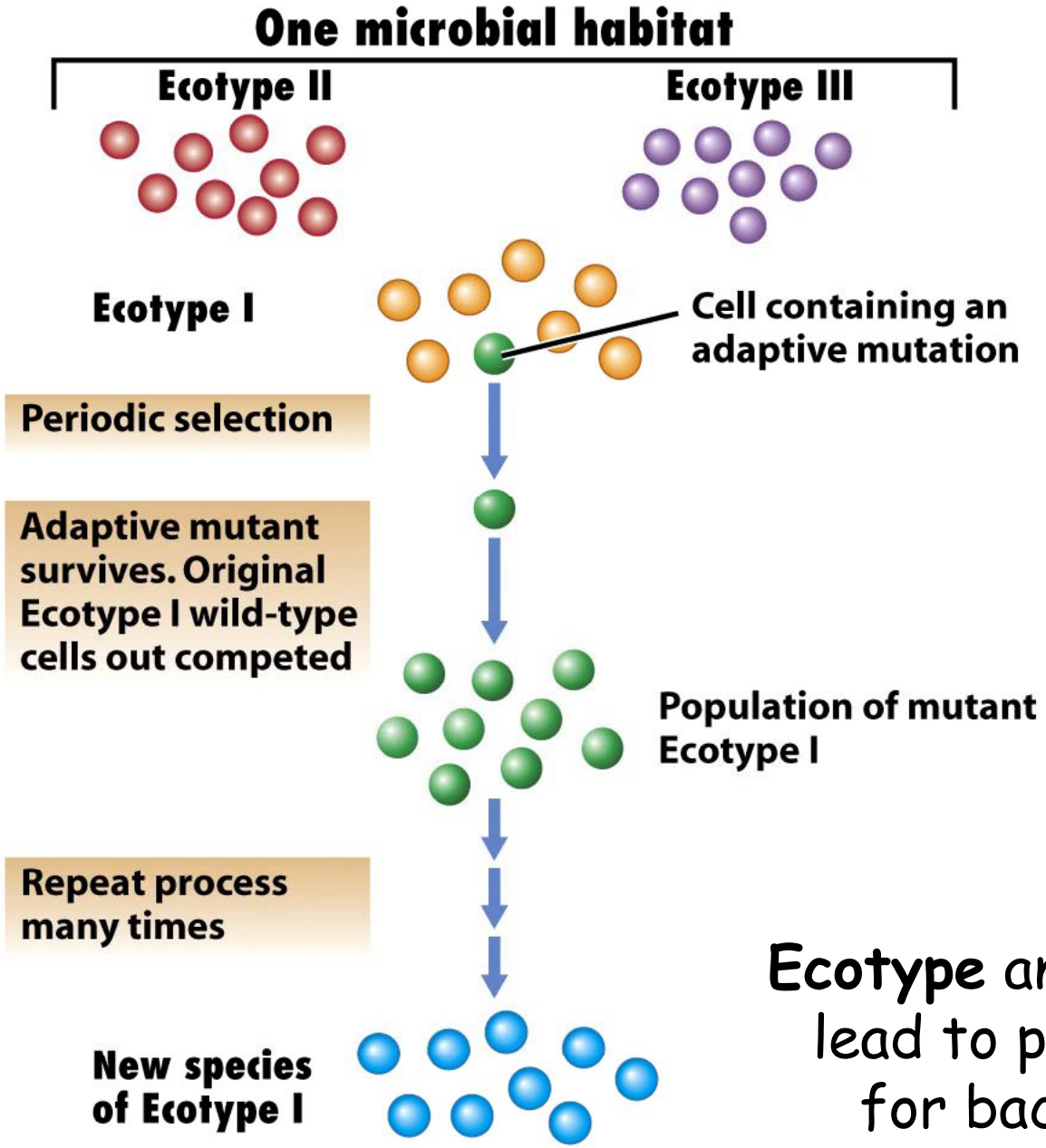
Species	C value (kb)
Navicola pelliculosa (diatom)	35,000
Drosophila melanogaster (fruitfly)	180,000
Paramecium aurelia (ciliate)	190,000
Gallus domesticus (chicken)	1,200,000
Erysiphe cichoracearum (fungus)	1,500,000
Cyprinus carpio (carp)	1,700,000
Lampreta planeri (lamprey)	1,900,000
Boa constrictor (snake)	2,100,000
Parascaris equorum (roundworm)	2,500,000
Carcarias obscurus (shark)	2,700,000
Rattus norvegicus (rat)	2,900,000
Xenopus laevis (toad)	3,100,000
***Homo sapiens* (human)**	**3,400,000**
Nicotiana tabaccum (tobacco)	3,800,000
Paramecium caudatum (ciliate)	8,600,000
Schistocerca gregaria (locust)	9,300,000
Allium cepa (onion)	18,000,000
Coscinodiscus asteromphalus (diatom)	25,000,000
Lilium formosanum (lily)	36,000,000
Pinus resinosa (pine)	68,000,000
Amphiuma means (newt)	84,000,000
Protopterus aethiopicus (lungfish)	140,000,000
Ophioglossum petiolatum (fern)	160,000,000
Amoeba proteus (amoeba)	290,000,000
Amoeba dubia (amoeba)	670,000,000

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.

Table 17.2 Comparison of *E. coli* and its primate host species[a]

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

[a]Adapted from J. T. Staley, *ASM News*, 1999.
[b]Value for all primates.
[c]Mouse 18S rRNA differs from humans by 16 bases.
[d]Comparison between *Homo sapiens* and chimpanzee.
[e]Comparison between *Homo sapiens* and lemurs.



Ecotype and **periodic selection** lead to possible mechanism for bacterial speciation.