

MICROBIOLOGIA GENERALE

Microbial evolution and systematics

**Microbial evolution and
systematics:**

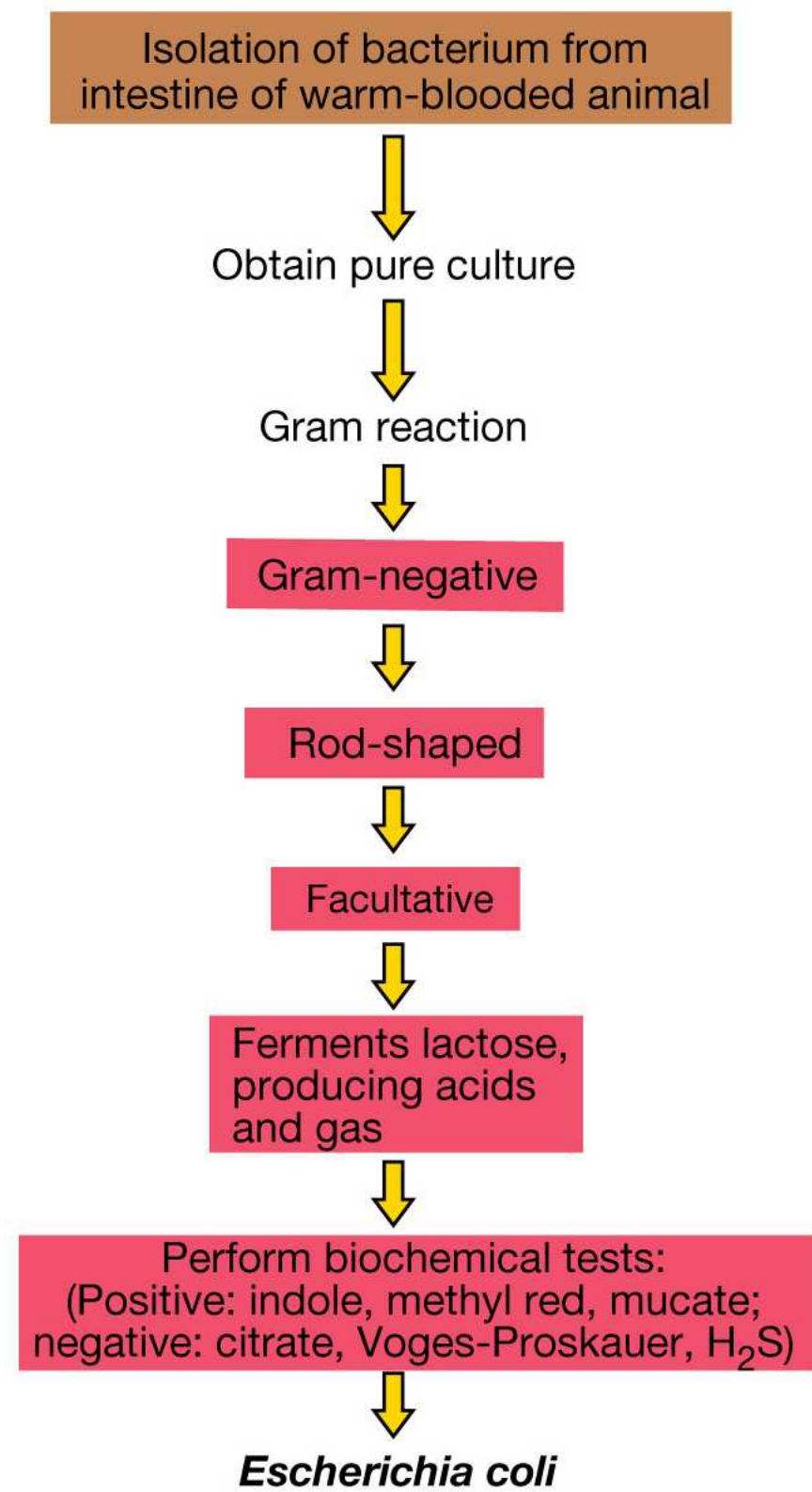
**conventional and molecular
taxonomy**

Tab. 11.4**Alcune caratteristiche fenotipiche di valore tassonomico**

Categoria principale	Componenti
Morfologia Motilità	Forma; dimensione; reazione Gram Motilità per mezzo di flagelli; motilità per scivolamento; motilità per mezzo di vescicole gassose; assenza di motilità
Nutrizione e fisiologia	Meccanismo di conservazione dell'energia (fototrofo, chemioorganotrofo, chemiolitotrofo); relazione con l'ossigeno; richiesta/tolleranza a temperatura, pH, e sale; capacità di usare varie fonti di carbonio, azoto e zolfo
Altri fattori	Pigmenti; inclusioni cellulari, o strati superficiali; patogenicità; sensibilità agli antibiotici

Conventional taxonomy

Example of methods that would be used for identification of a newly isolated enteric bacterium, using classic microbiological methods



I. Isolamento e microscopia

Isolamento



Coltura pura



Colorazione di Gram/morfologia

II. Fisiologia generale

Bastoncello
Gram-negativo



Facoltativo



Fermenta il lattosio,
con produzione
di acidi e gas

III. Fisiologia dettagliata

Facoltativa
fermentazione
del lattosio



Esegue serie
di prove
biochimiche



Positivi:
indolo, rosso
metile e mucato
Negativi:
citrato,
Voges-Proskauer, H₂S

IV. Conclusione



Escherichia coli

Ranges of GC ratios of various organisms

Organism

Prokaryotes

Bacteria

Archaea

Eukaryotes

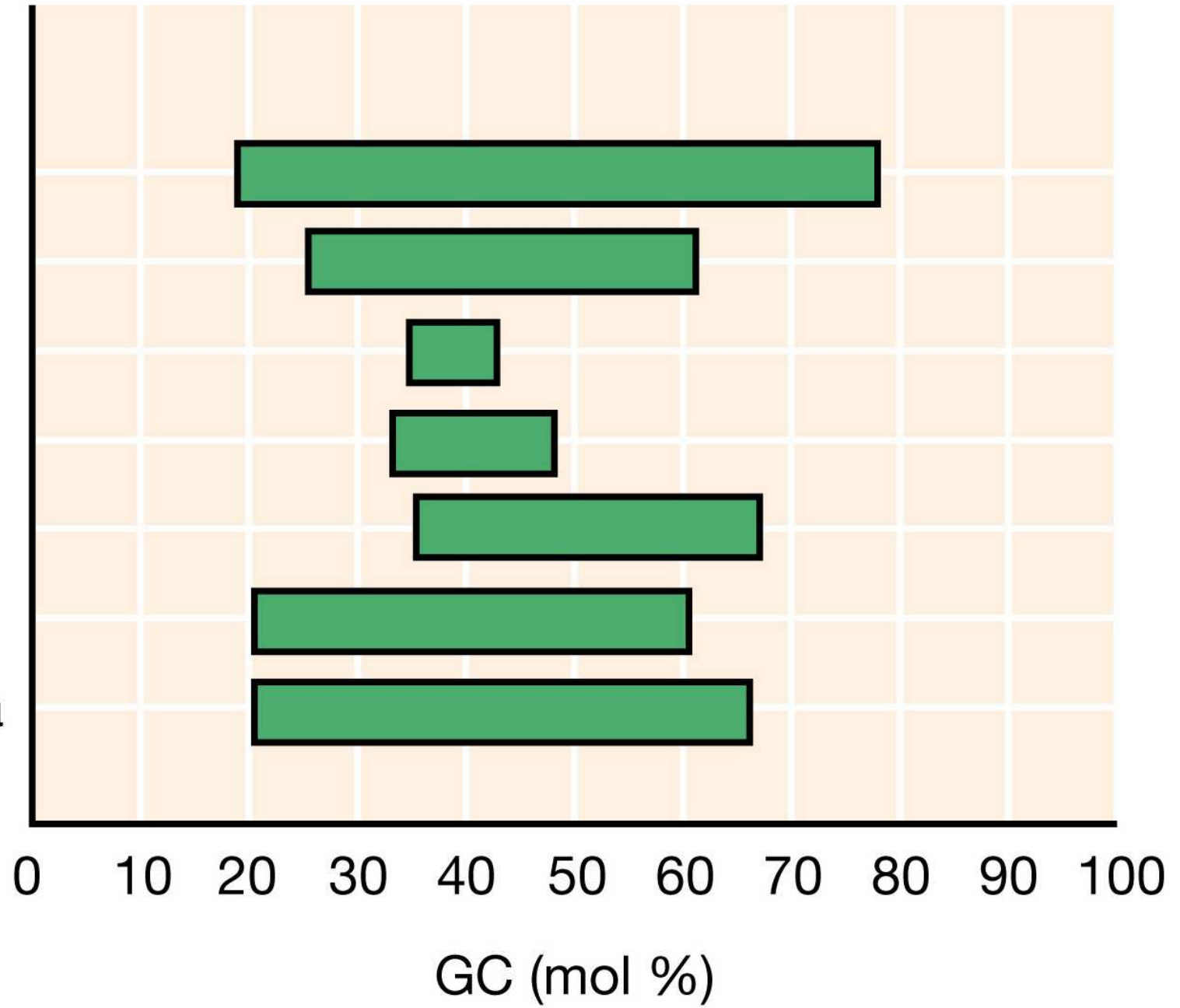
Animals

Plants

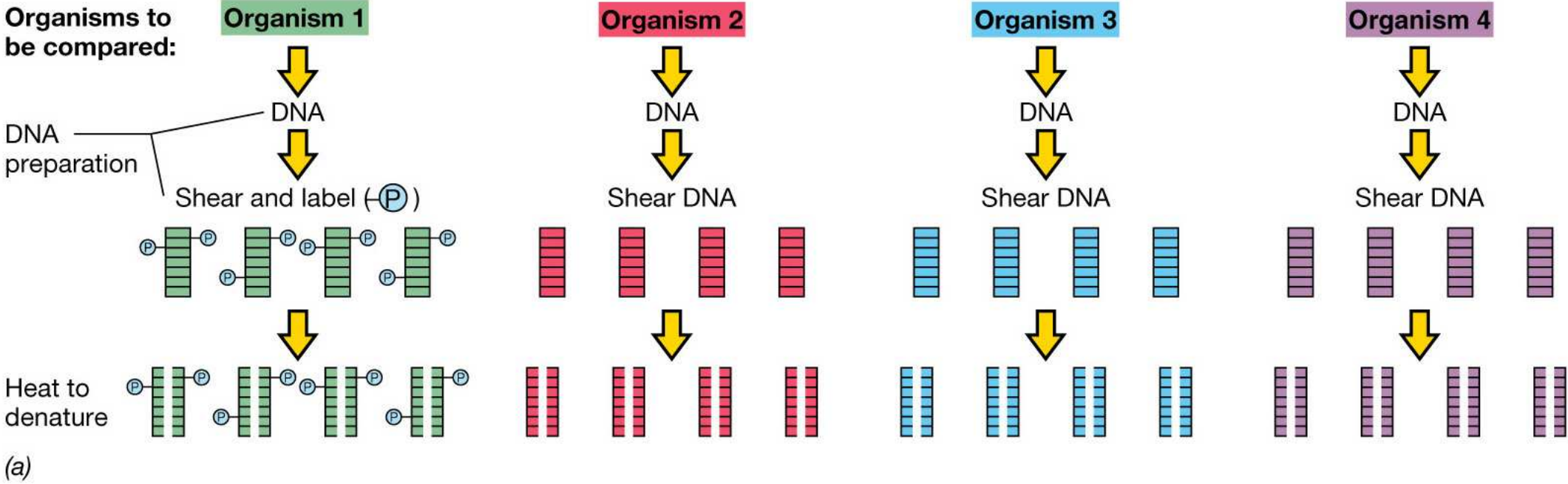
Algae

Fungi

Protozoa



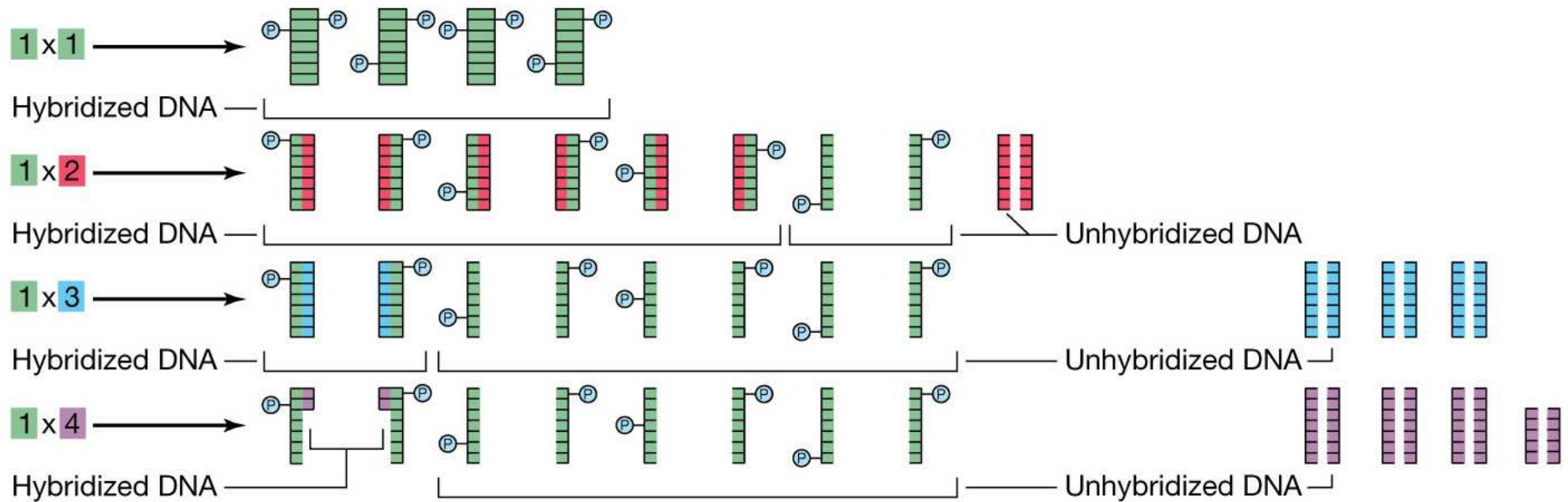
Genomic hybridization as a taxonomic tool



Genomic hybridization as a taxonomic tool

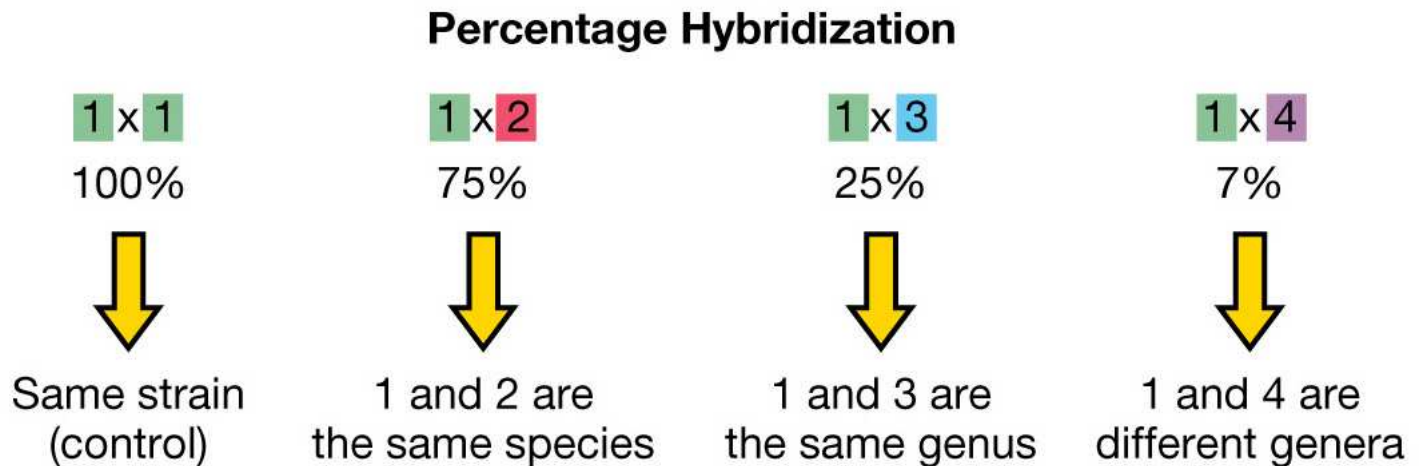
Hybridization

experiment: Mix DNA from two organisms—unlabeled DNA is added in excess:



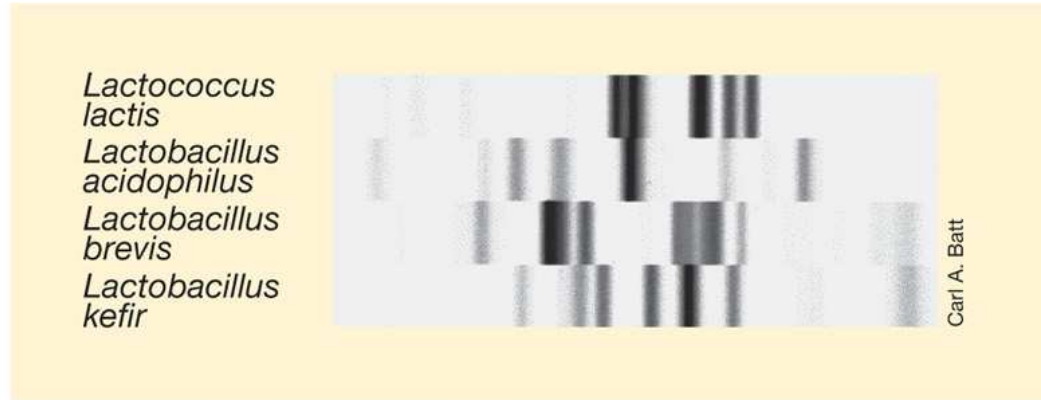
(b)

Results and interpretation:

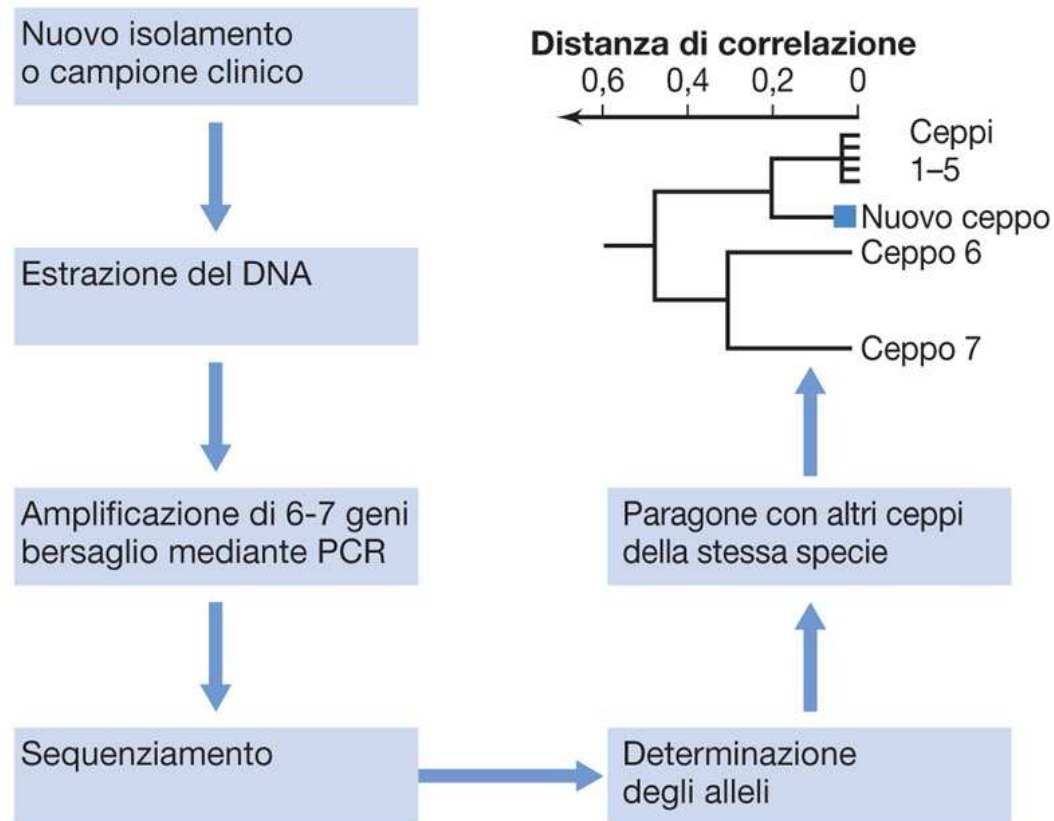


(c)

Ribotyping



(a)



(b) Multilocus sequence typing (MLST)

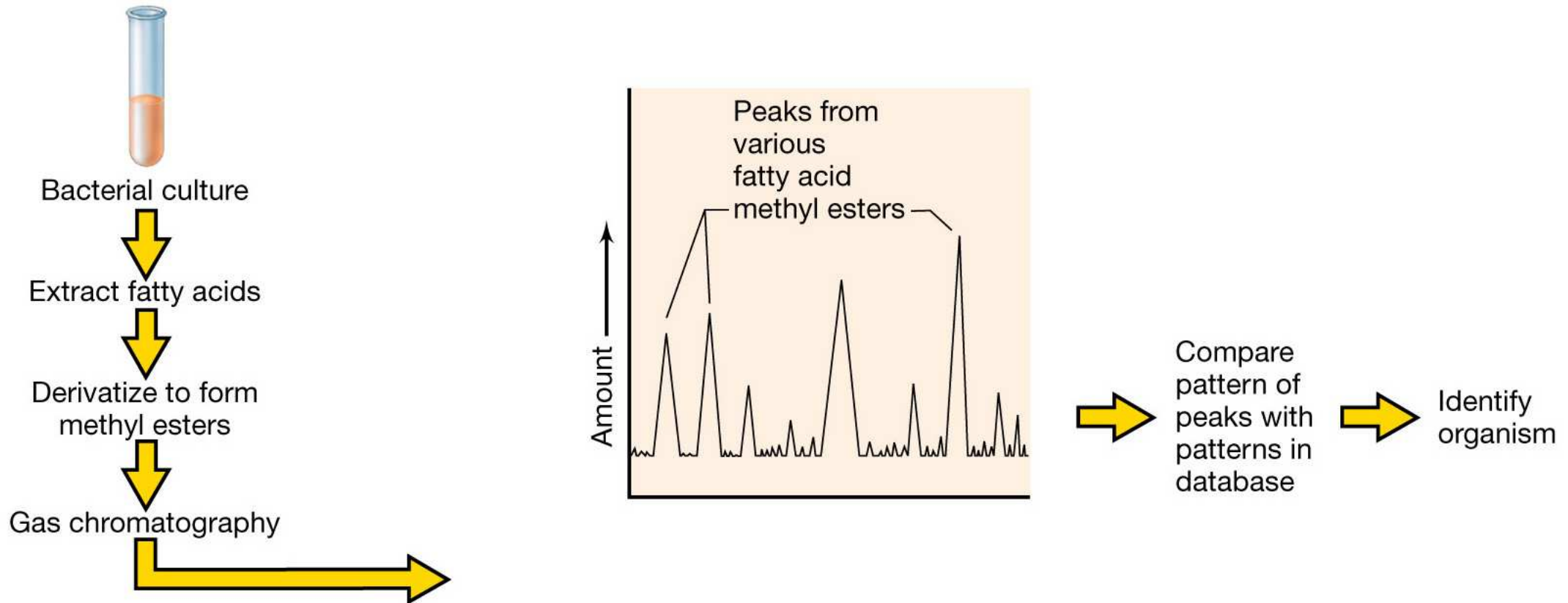
Fatty acid methyl ester (FAME) analysis in bacterial identification: classes of fatty acids in *Bacteria*.

Classes of Fatty Acids in *Bacteria*

Class	Example	Structure of example
Saturated	tetradecanoic acid	$\begin{array}{c} \text{O} \\ \parallel \\ \text{HO}-\text{C}-(\text{CH}_2)_{12}-\text{CH}_3 \end{array}$
Unsaturated	<i>omega</i> -7- <i>cis</i> hexadecanoic acid	$\begin{array}{c} \text{O} \qquad \qquad \text{H} \quad \text{H} \\ \parallel \qquad \qquad \diagup \quad \diagdown \\ \text{HO}-\text{C}-(\text{CH}_2)_6-\text{C}=\text{C}-(\text{CH}_2)_6-\text{CH}_3 \\ \qquad \qquad \qquad \diagdown \quad \diagup \end{array}$
Cyclopropane	<i>cis</i> 7-8 methylene hexadecanoic acid	$\begin{array}{c} \text{O} \qquad \qquad \text{H} \quad \text{H} \\ \parallel \qquad \qquad \diagup \quad \diagdown \\ \text{HO}-\text{C}-(\text{CH}_2)_7-\text{C}=\text{C}-(\text{CH}_2)_5-\text{CH}_3 \\ \qquad \qquad \qquad \diagdown \quad \diagup \\ \qquad \qquad \qquad \text{C} \\ \qquad \qquad \qquad \diagup \quad \diagdown \\ \qquad \qquad \qquad \text{H} \quad \text{H} \end{array}$
Branched	13-methyltetradecanoic acid	$\begin{array}{c} \text{O} \qquad \qquad \text{CH}_3 \\ \parallel \qquad \qquad \\ \text{HO}-\text{C}-(\text{CH}_2)_{10}-\text{C}-\text{CH}_3 \\ \qquad \qquad \qquad \\ \qquad \qquad \qquad \text{H} \end{array}$
Hydroxy	3-hydroxytetradecanoic acid	$\begin{array}{c} \text{O} \qquad \qquad \text{H} \\ \parallel \qquad \qquad \\ \text{HO}-\text{C}-\text{CH}_2-\text{C}-(\text{CH}_2)_{10}-\text{CH}_3 \\ \qquad \qquad \qquad \\ \qquad \qquad \qquad \text{OH} \end{array}$

(a)

Fatty acid methyl ester (FAME) analysis in bacterial identification

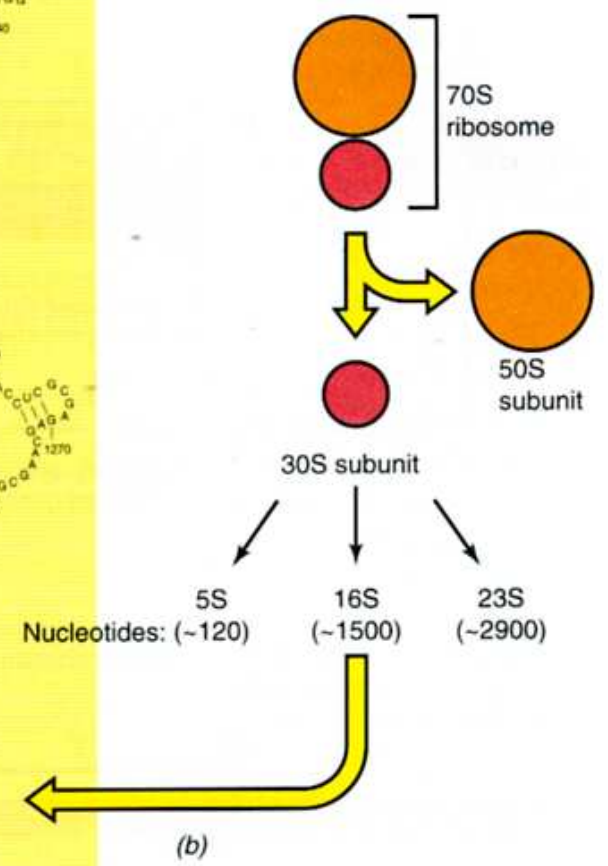
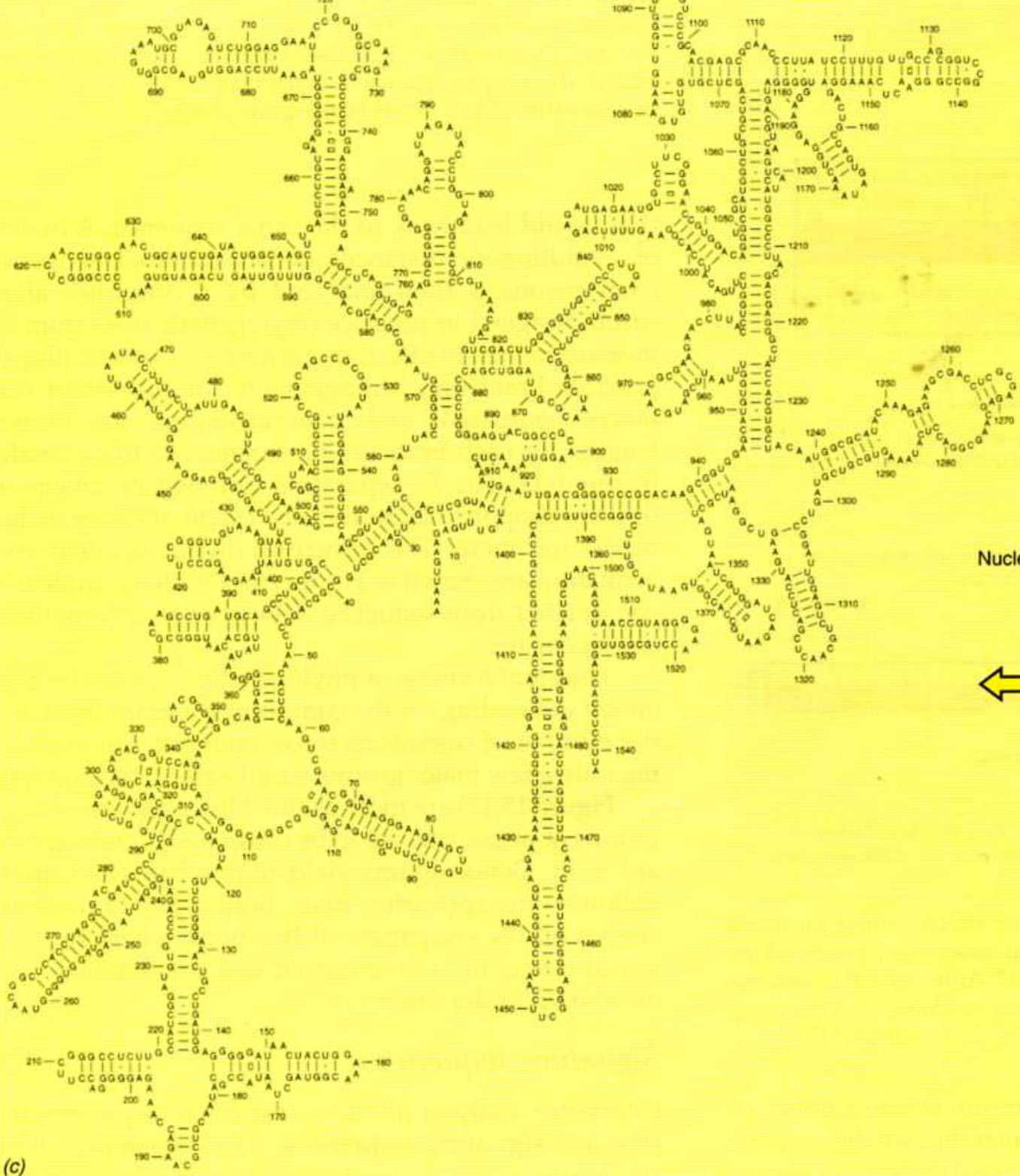


(b)

Each peak from the gas chromatograph is due to one particular fatty acid methyl ester and the peak height is proportional to the amount.

**Microbial evolution and
systematics:**

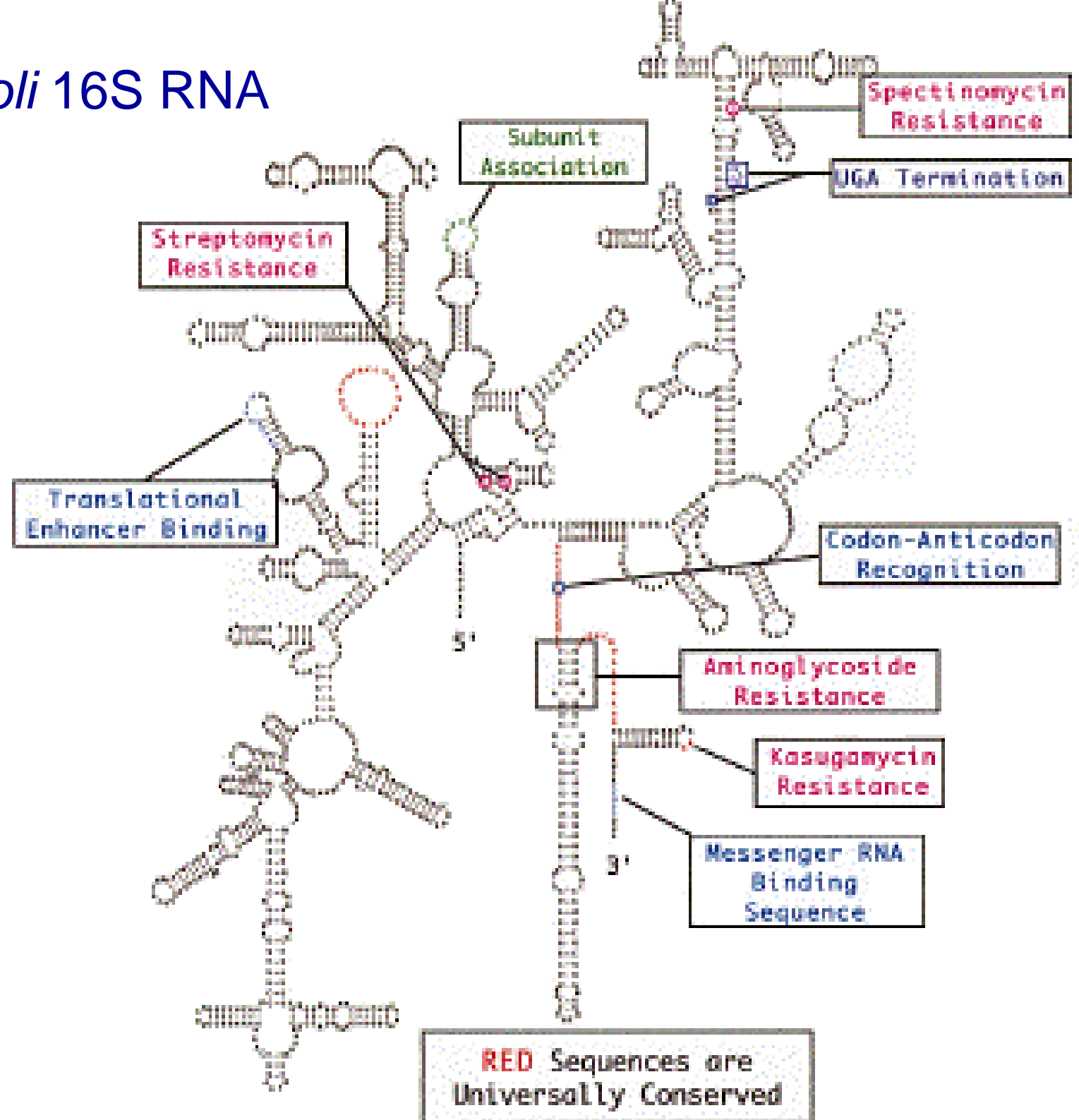
**evolutionary chronometers
and microbial phylogeny**



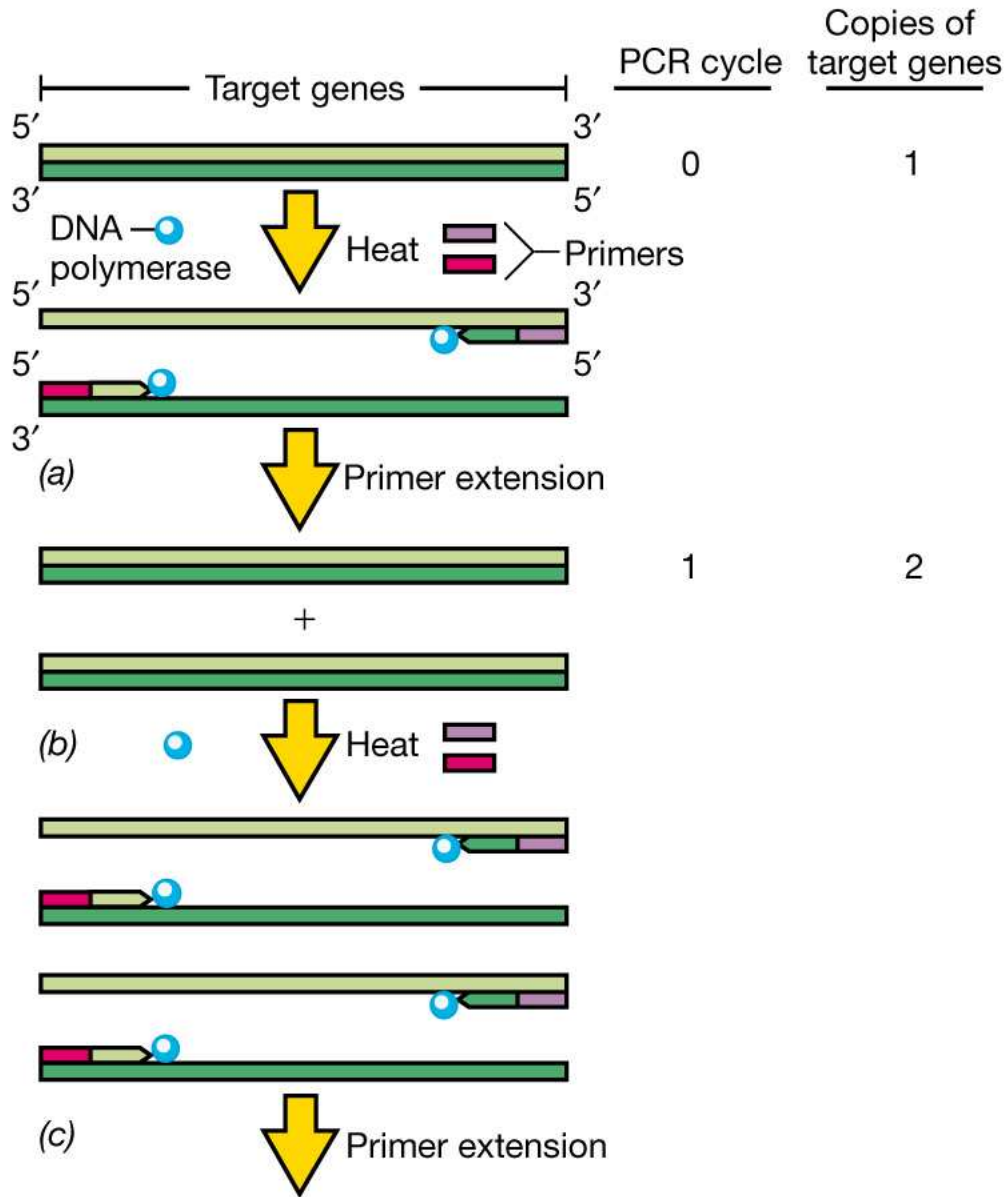
Escherichia coli
16S RNA

(c)

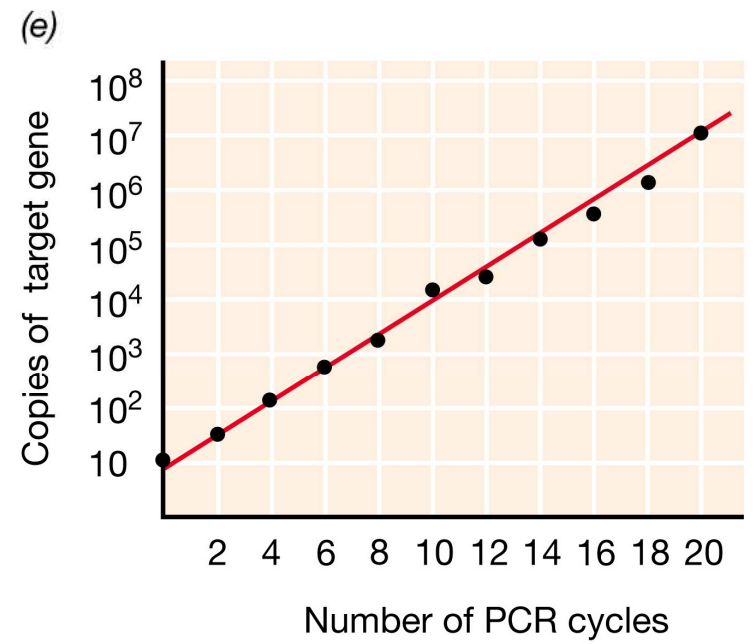
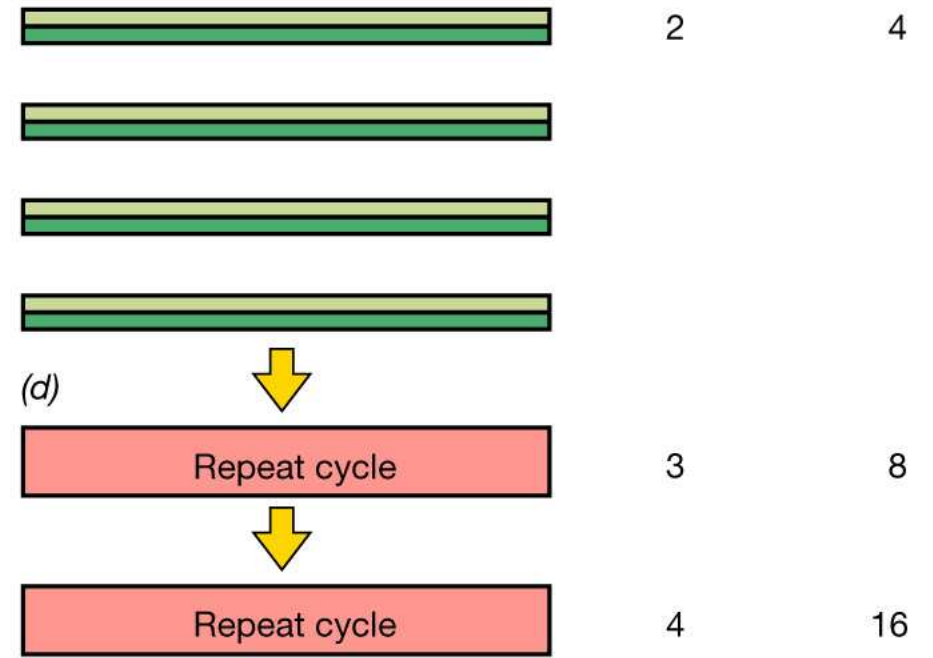
Escherichia coli 16S RNA



The polymerase chain reaction (PCR)



PCR cycle	Copies of target genes
0	1
1	2
2	4
3	8
4	16

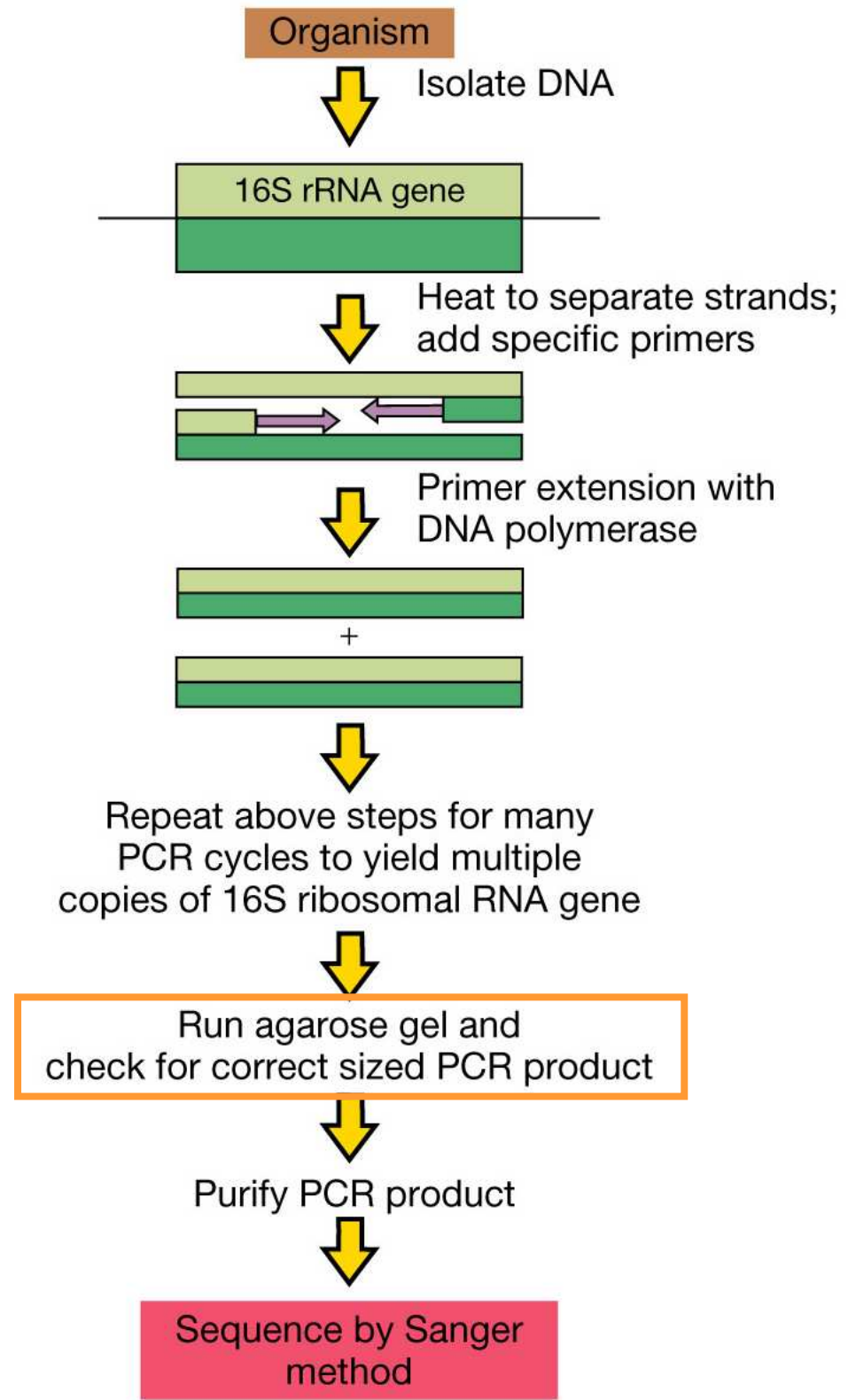


(f)

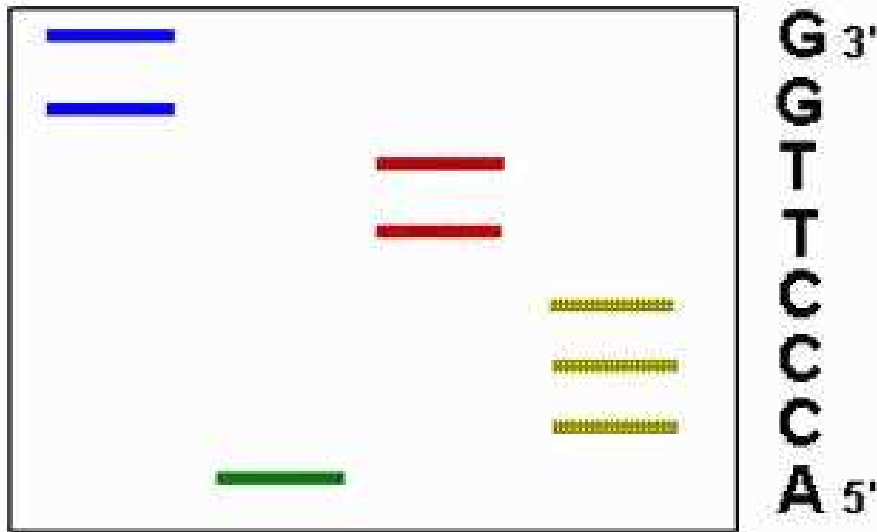
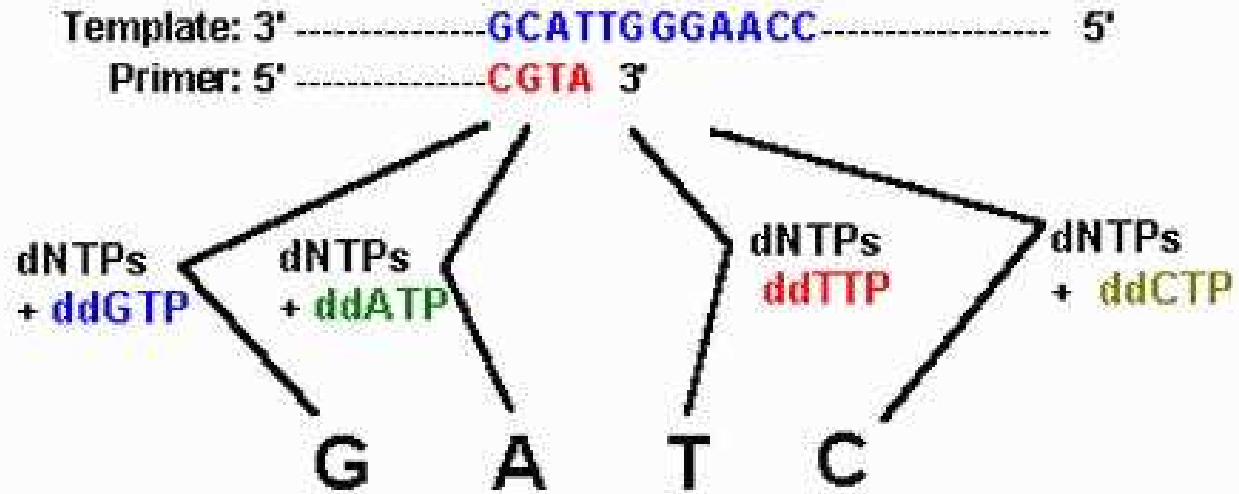
Ribosomal RNA sequencing of a pure culture of a microorganism using the PCR

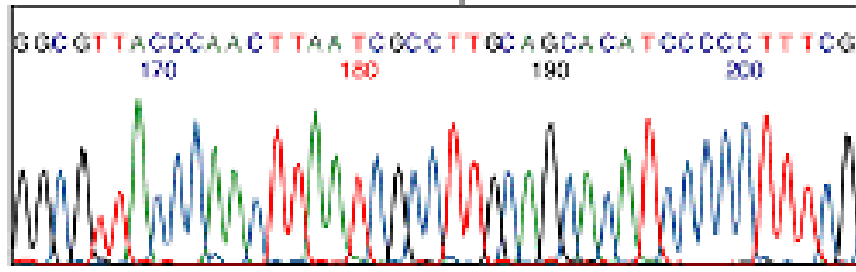
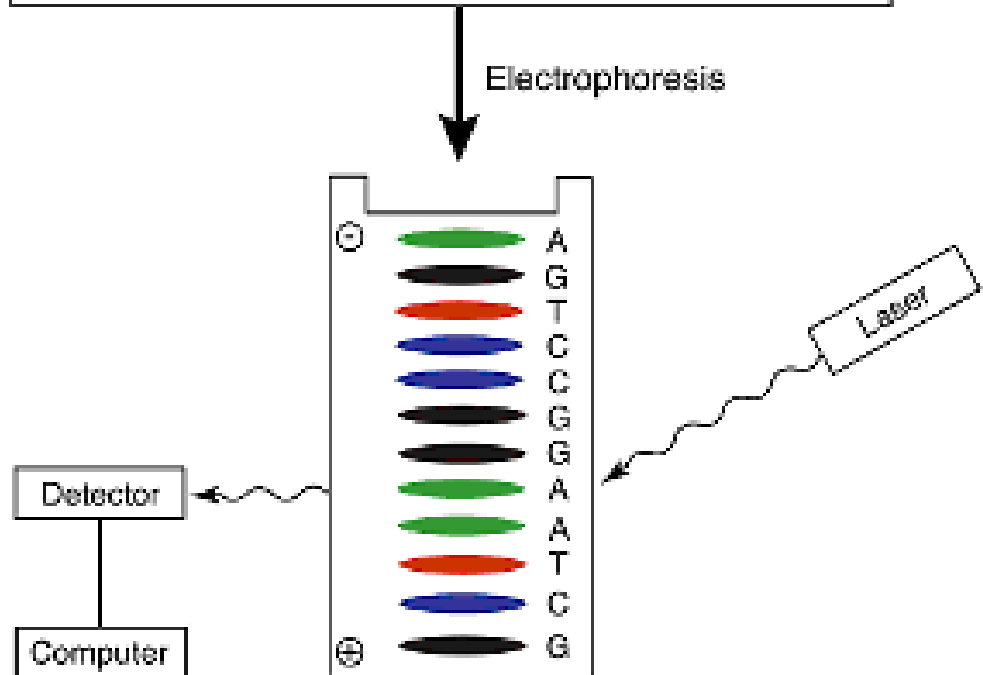
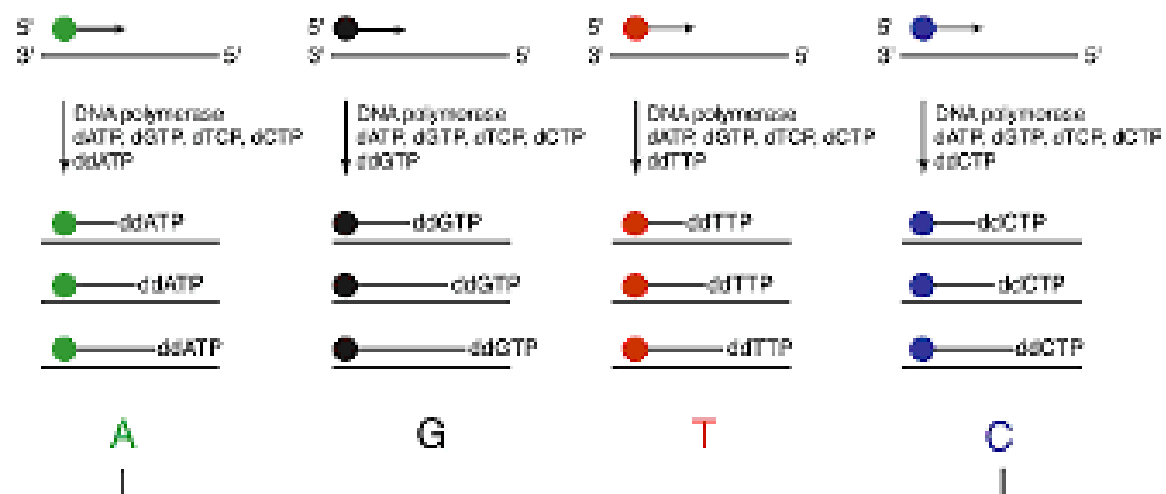


16S-ribosomal RNA genes located on the *E. coli* genome amplified by PCR



Sanger ddNTP chain terminator sequencing

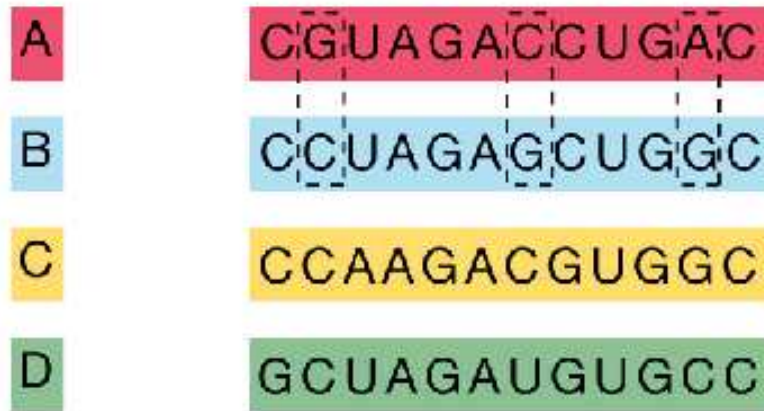




Organism

Sequence

Analysis



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

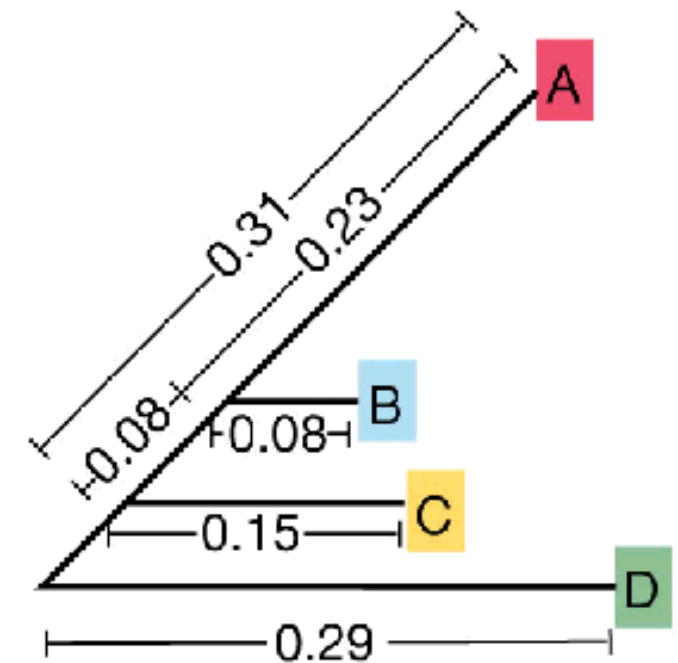
Preparing a phylogenetic distance tree from 16S rRNA sequences

(a) Sequence alignment and analysis

Evolutionary distance

Corrected evolution

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

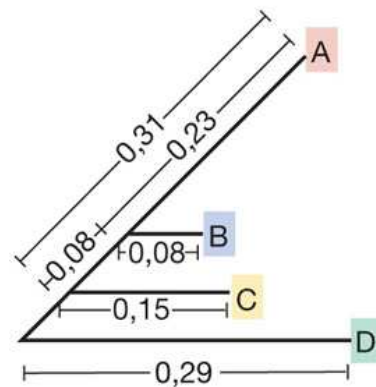
(c) Phylogenetic tree

Organismo	Sequenza	Analisi
A	C G U A G A C C U G A C	Per A → B, esistono tre differenze su un totale di dodici; quindi $\frac{3}{12} = 0,25$
B	C C U A G A G C U G G C	
C	C C A A G A C G U G G C	
D	G C U A G A U G U G C C	

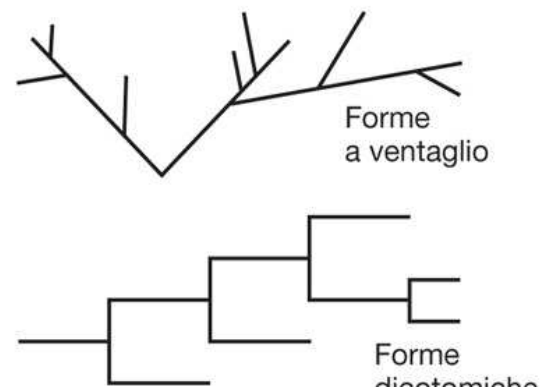
(a) Allineamento e analisi della sequenza

	Distanza evolutiva	Distanza evolutiva corretta
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) Calcolo della distanza evolutiva



(c) Albero filogenetico



(d) Topologie dell'albero

Tab. 11.1 Sequenze tipizzanti negli rRNA 16S o 18S che caratterizzano i tre domini degli organismi viventi

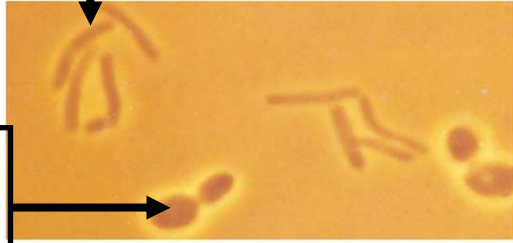
Oligonucleotide tipizzante ^a	Posizione approssimativa ^b	Frequenza di comparsa ^c		
		Archea	Batteri	Eucarioti
CACYYG	315	0	>95	0
AAACUCAA	910	3	100	0
AAACUUAAG	910	100	0	100
YUYAAUUG	960	100	<1	100
CAACCYCR	1110	0	>95	0
UCCCUG	1380	>95	0	100
UACACACCG	1400	0	>99	100
CACACACCG	1400	100	0	0

^a Y, qualsiasi pirimidina; R, qualsiasi purina.

^b Fare riferimento alla figura 11.11c per lo schema di numerazione dell'rRNA 16S.

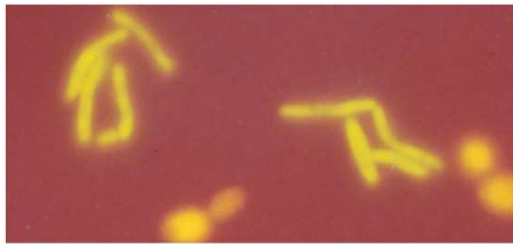
^c La comparsa si riferisce alla percentuale di organismi esaminati in ciascun dominio contenente la sequenza.

Bacillus megaterium

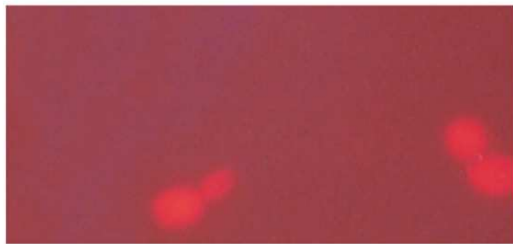


Saccharomyces cerevisiae

(a)

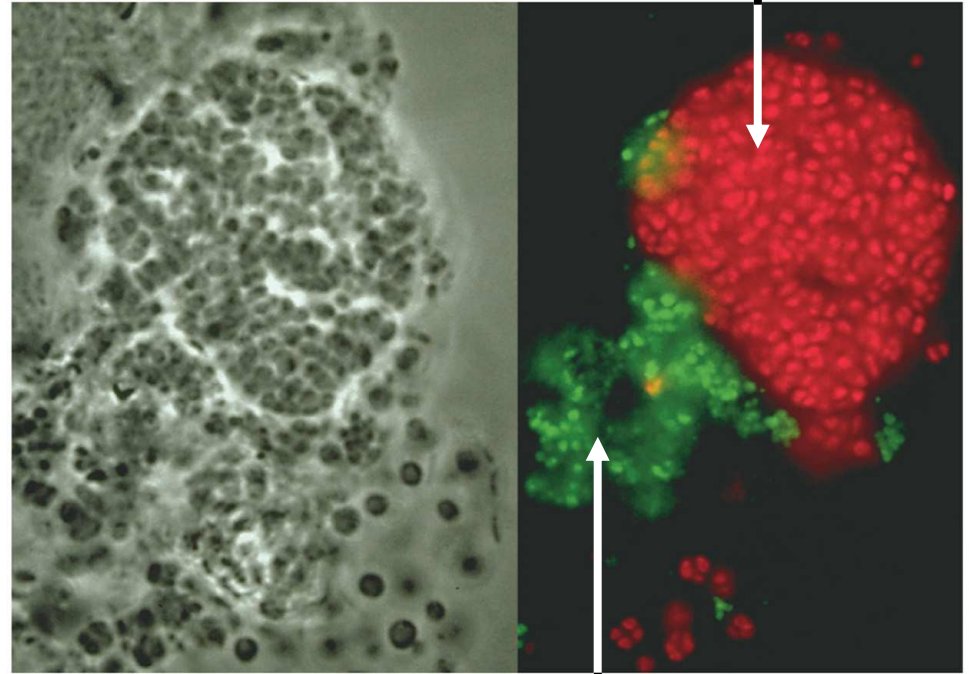


(b)



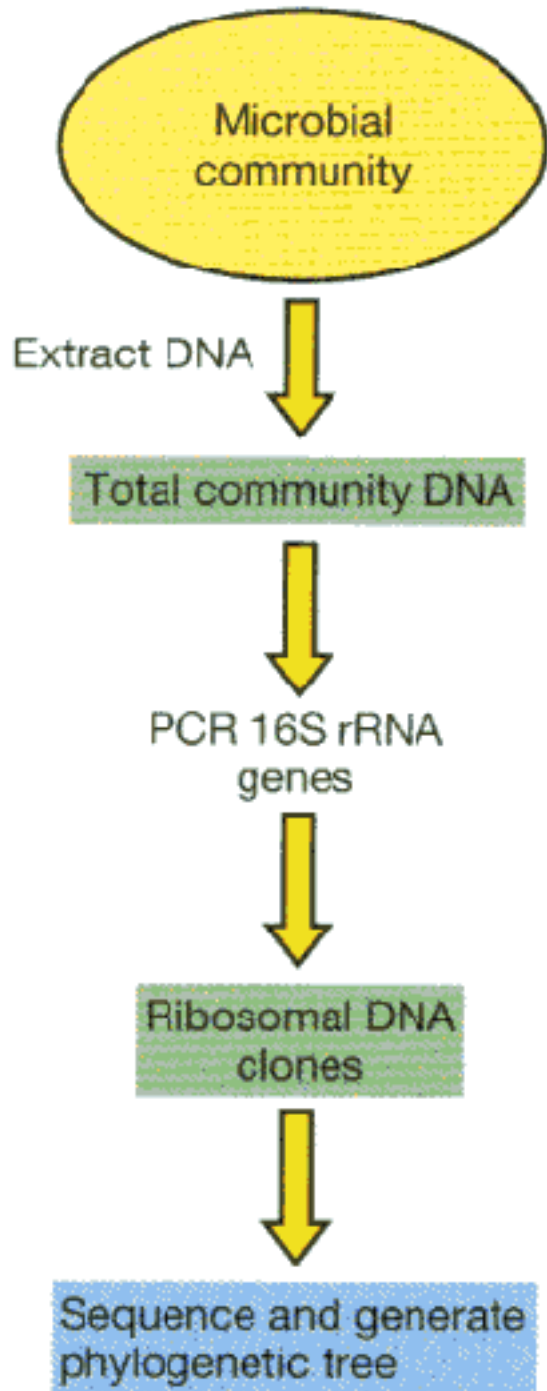
(c)

Nitrosomonas spp.



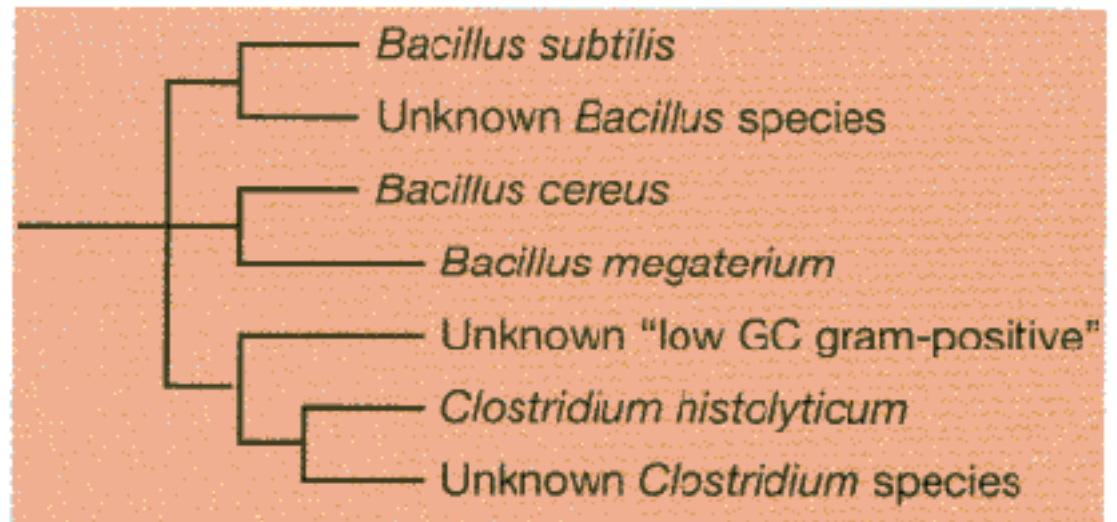
Nitrobacter spp.

David A. Stahl



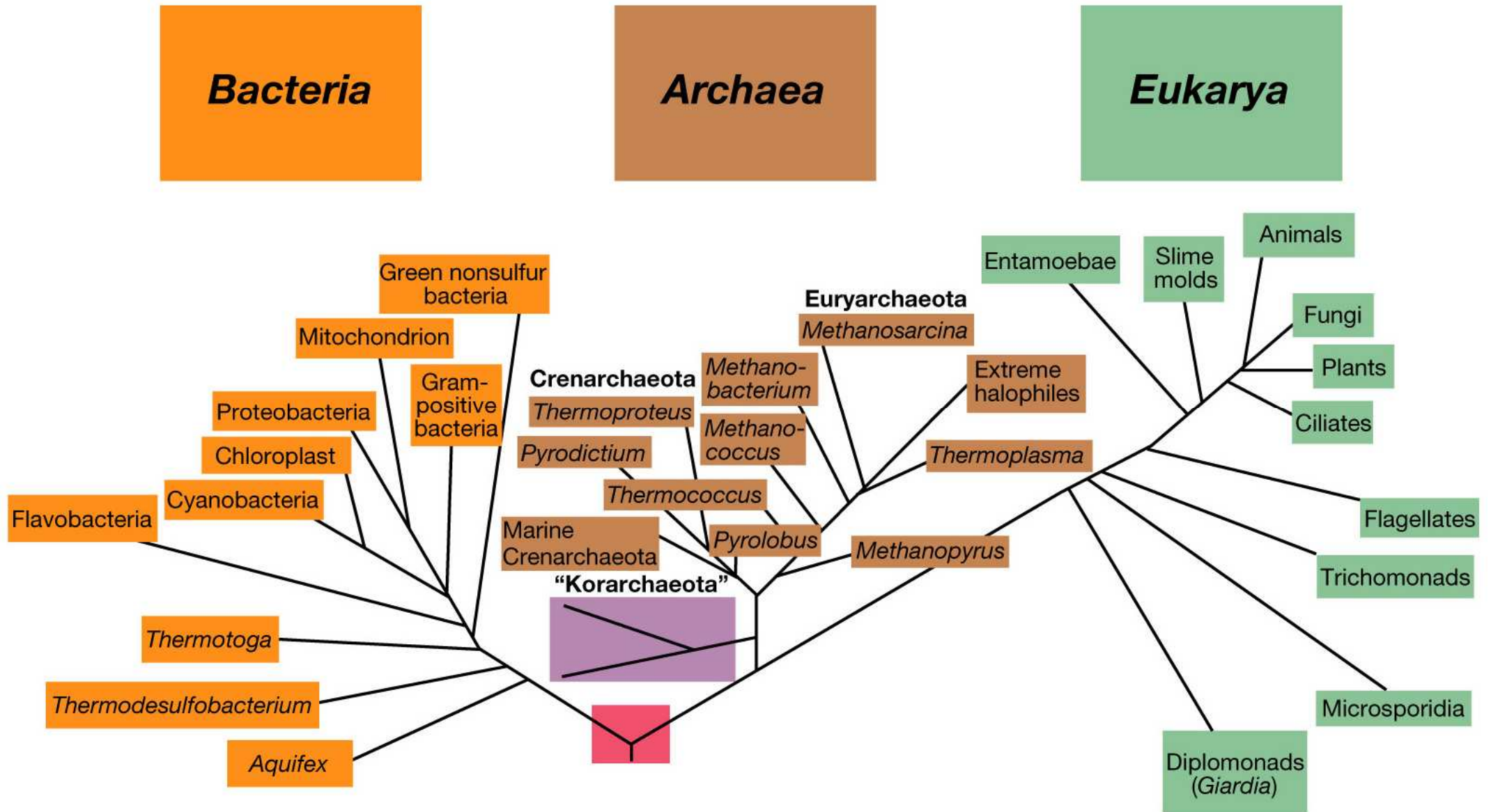
(a)

Steps in biodiversity analysis of a microbial community using phylogenetic trees



(b)

Universal phylogenetic tree as determined from comparative rRNA sequencing



**Microbial evolution and
systematics:**

**unique phenotypic characteristics
of the primary domains**

1: Cell walls

- Virtually all **Bacteria** have cell walls containing **peptidoglycan**.
- Peptidoglycan can thus be considered a signature for Bacteria

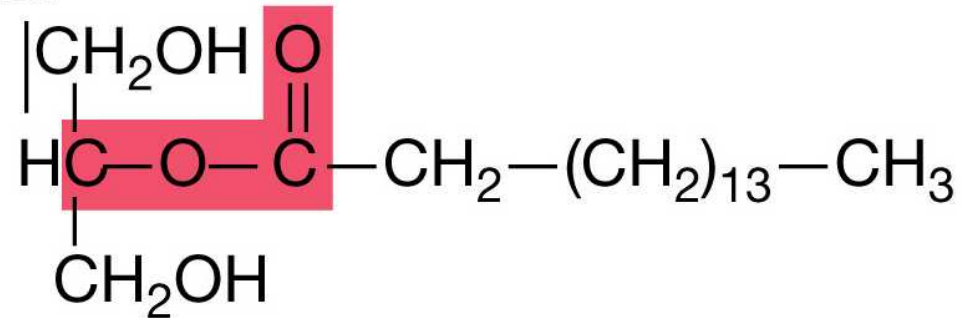
- Eukarya and Archaea lack peptidoglycan.

- In **Archaea** various cell wall types exist: pseudopeptidoglycan, polysaccharide, protein, or glycoprotein

- In **Eukarya** when present cell walls are made of **cellulose** or **chitin**

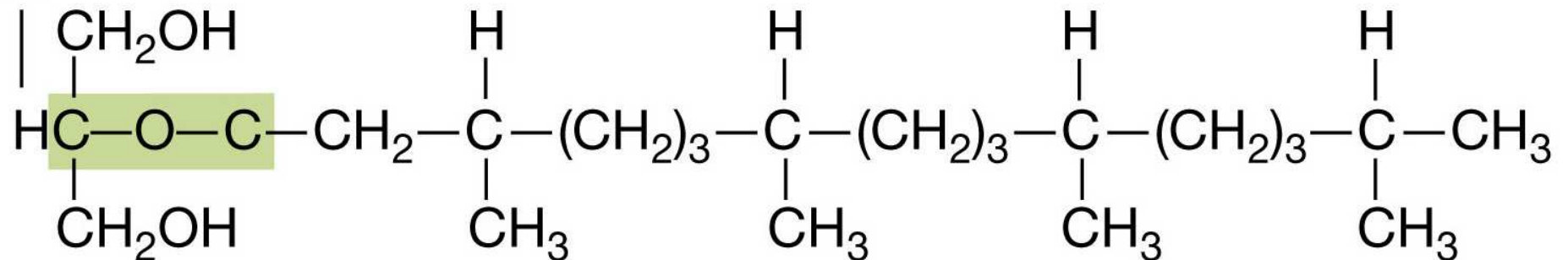
2: Plasma membrane lipids

Ester

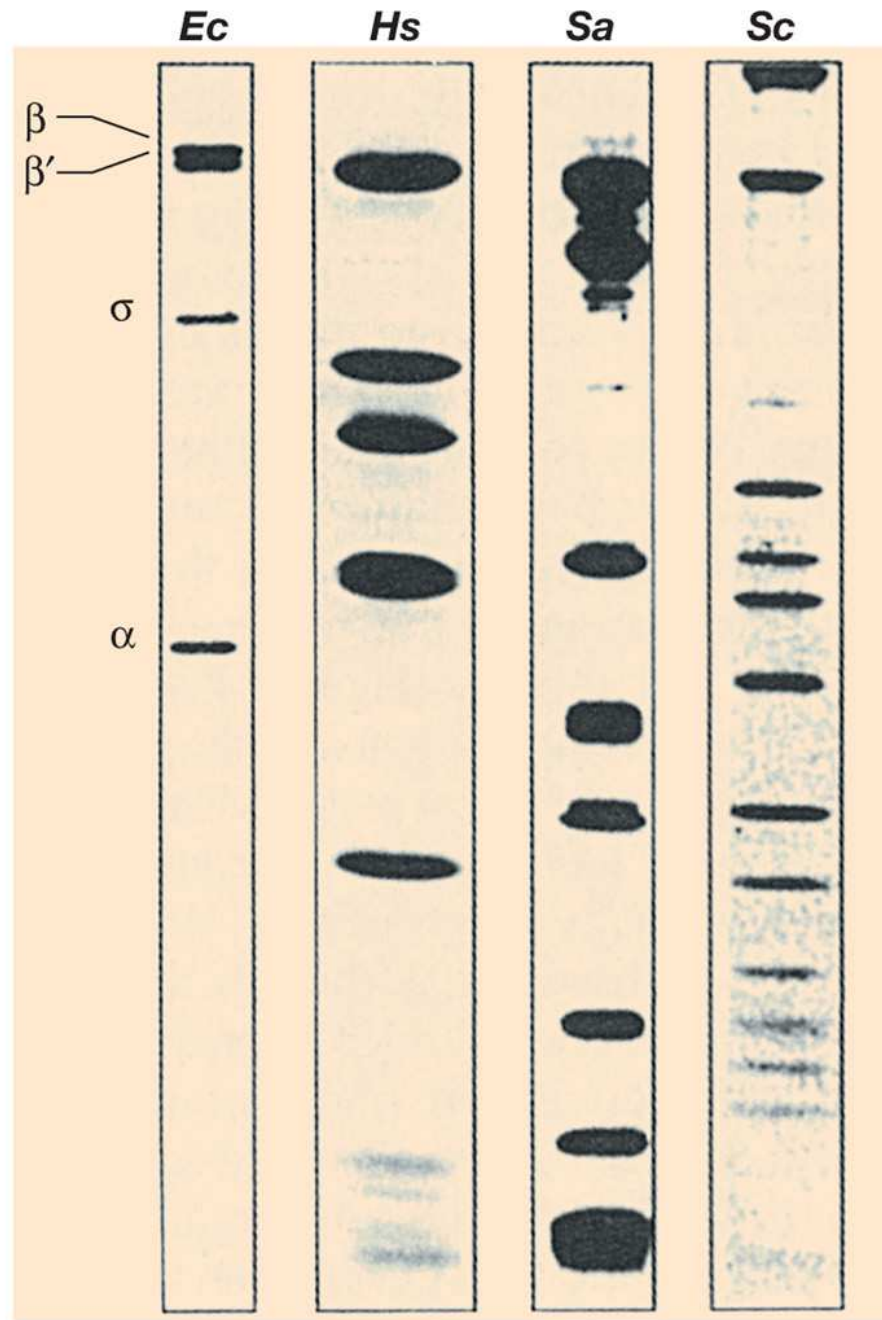


Bacteria, Eukarya

Ether



Archaea



W. Zillig

3: RNA polymerases

Tab. 11.3 Principali caratteristiche distintive di Batteri, Archea ed Eucarioti^a

Caratteristica	Batteri	Archea	Eucarioti
Morfologica e genetica			
Struttura cellulare procariotica	Sì	Sì	No
DNA presente in forma circolare chiusa covalentemente	Sì	Sì	No
Presenza di proteine istoniche	No	Sì	Sì
Nucleo racchiuso da membrana	Assente	Assente	Presente
Parete cellulare	Acido muramico presente	Acido muramico assente	Acido muramico assente
Lipidi di membrana	Legame estere	Legame etere	Legame estere
Ribosomi (massa)	70S	70S	80S
tRNA iniziatore	Formilmetionina	Metionina	Metionina
Introni nella maggior parte dei geni	No	No	Sì
Operoni	Sì	Sì	No
Capping e code di poli-A negli mRNA	No	No	Sì
Plasmidi	Sì	Sì	Rari
Sensibilità dei ribosomi alla tossina difterica	No	Sì	Sì
RNA polimerasi (<i>vedi</i> fig. 11.19)	Una (4 subunità)	Diverse (ciascuna di 8-12 subunità)	Tre (ciascuna di 12-14 subunità)
Richiesta di fattori di trascrizione (► cap. 7.11)	No	Sì	Sì
Struttura del promotore (► cap. 7.10 e 7.11)	Sequenze -10 e -35 (Pribnow box)	TATA box	TATA box
Sensibilità a cloramfenicolo, streptomina e kanamicina	Sì	No	No
Fisiologica			
Metanogenesi	No	Sì	No
Riduzione dissimilativa di S ⁰ o SO ₄ ²⁻ a H ₂ S, o Fe ³⁺ a Fe ²⁺	Sì	Sì	No
Nitrificazione	Sì	No ^b	No
Denitrificazione	Sì	Sì	No
Fissazione dell'azoto	Sì	Sì	No
Fotosintesi clorofilliana	Sì	No	Sì (nei cloroplasti)
Metabolismo energetico basato sulla rodopsina	Sì	Sì	No
Chemiolitotrofia (Fe, S, H ₂)	Sì	Sì	No
Vescicole di gas	Sì	Sì	No
Sintesi di granuli di deposito di carbonio costituiti da β-idrossialcanoati	Sì	Sì	No
Crescita al di sopra di 80 °C	Sì	Sì	No
Crescita al di sopra di 100 °C	No	Sì	No

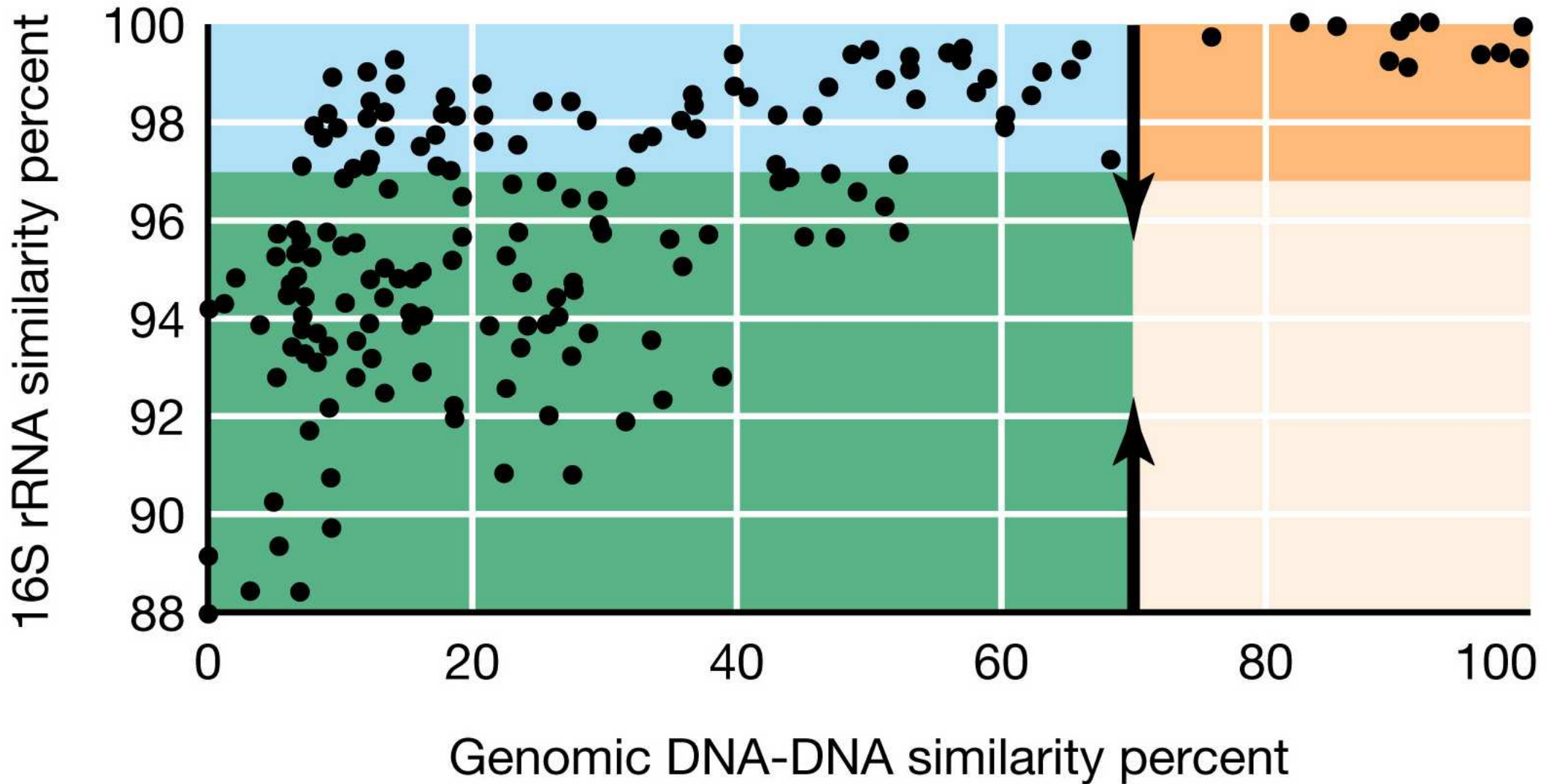
^a Si noti che per molte caratteristiche solo particolari rappresentanti di un dato dominio mostrano la proprietà in questione.

^b Studi genomici ambientali di procarioti in acque marine indicano chiaramente che esistono Archea nitrificanti (► vol. 2A, cap. 19.6)

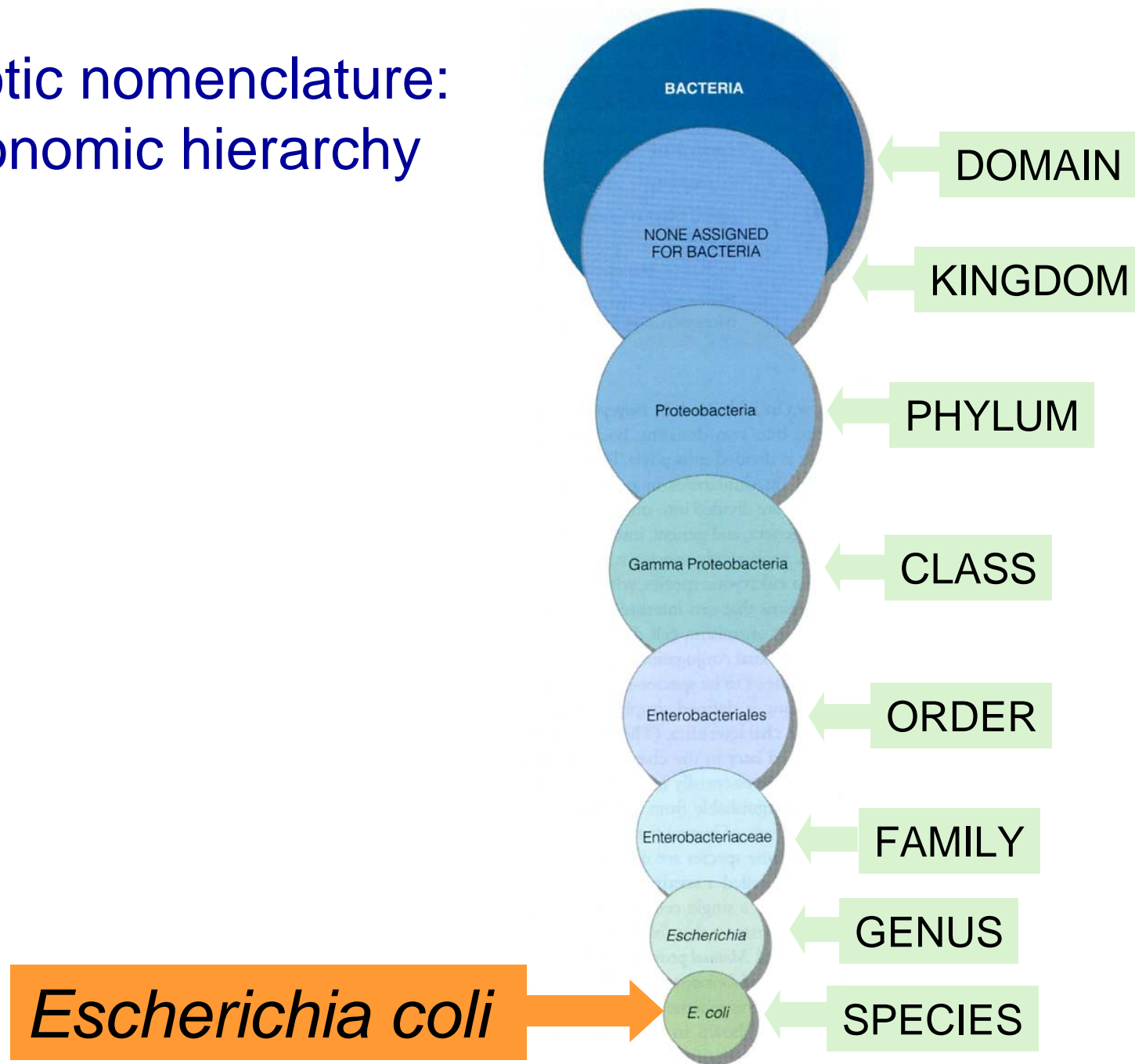
**Microbial evolution and
systematics:**

**the specie concept in
microbiology and
nomenclature**

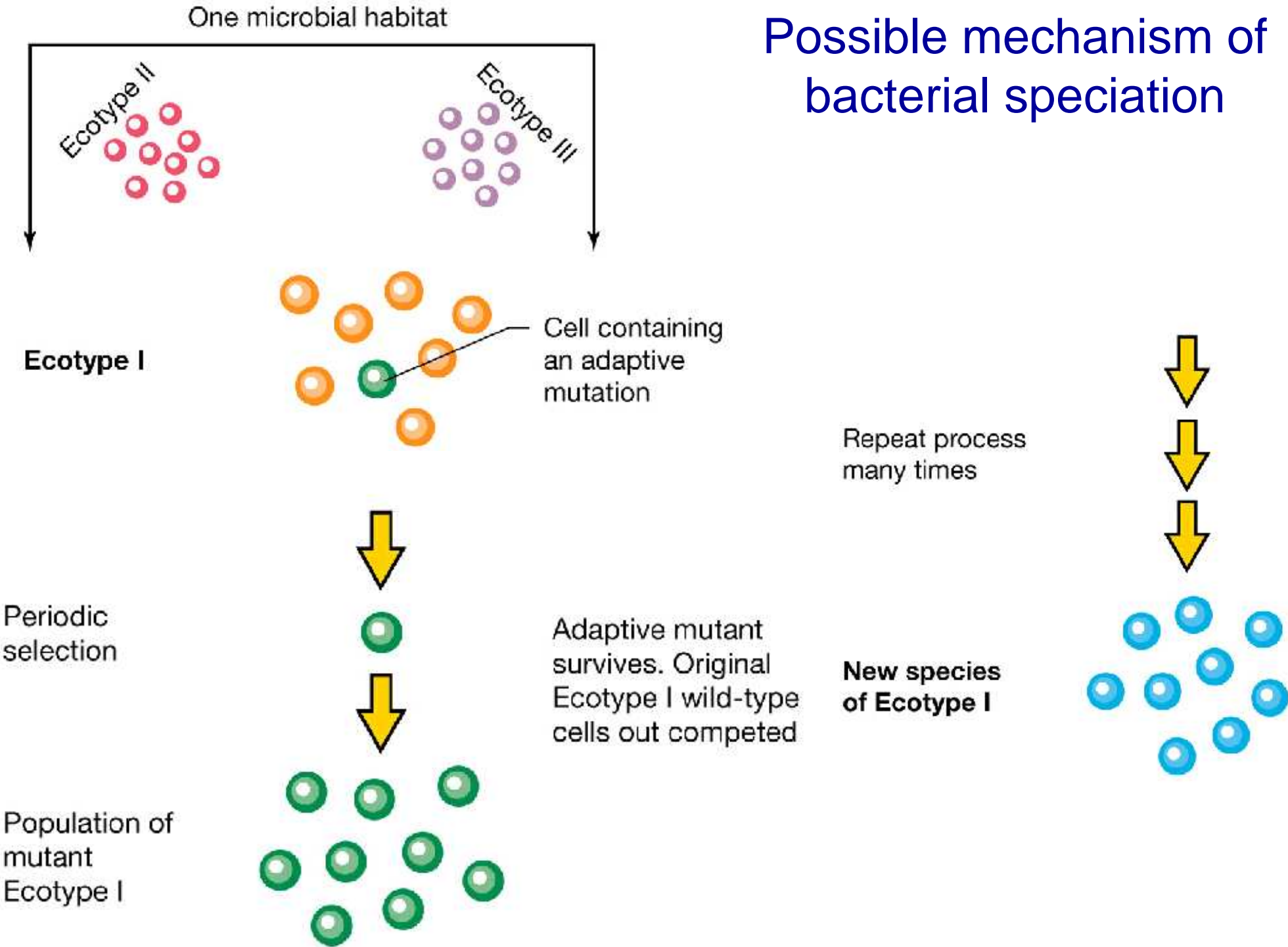
Relationship between 16S ribosomal RNA sequence similarity and genomic DNA hybridization between different pairs of organisms



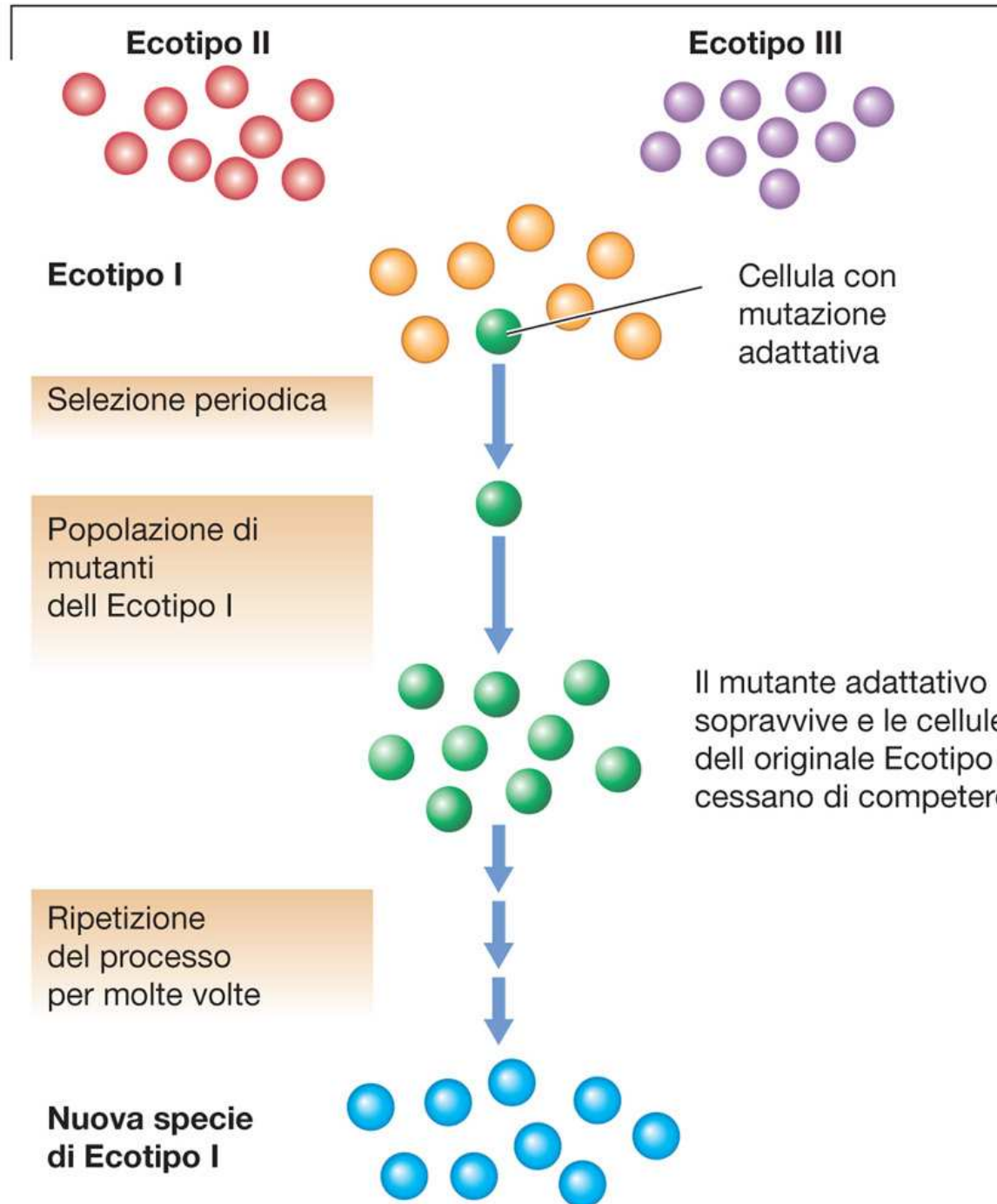
Prokaryotic nomenclature: the taxonomic hierarchy



Possible mechanism of bacterial speciation



Habitat microbico



Tab. 11.6**Livelli tassonomici e numeri delle specie procariotiche conosciute^a**

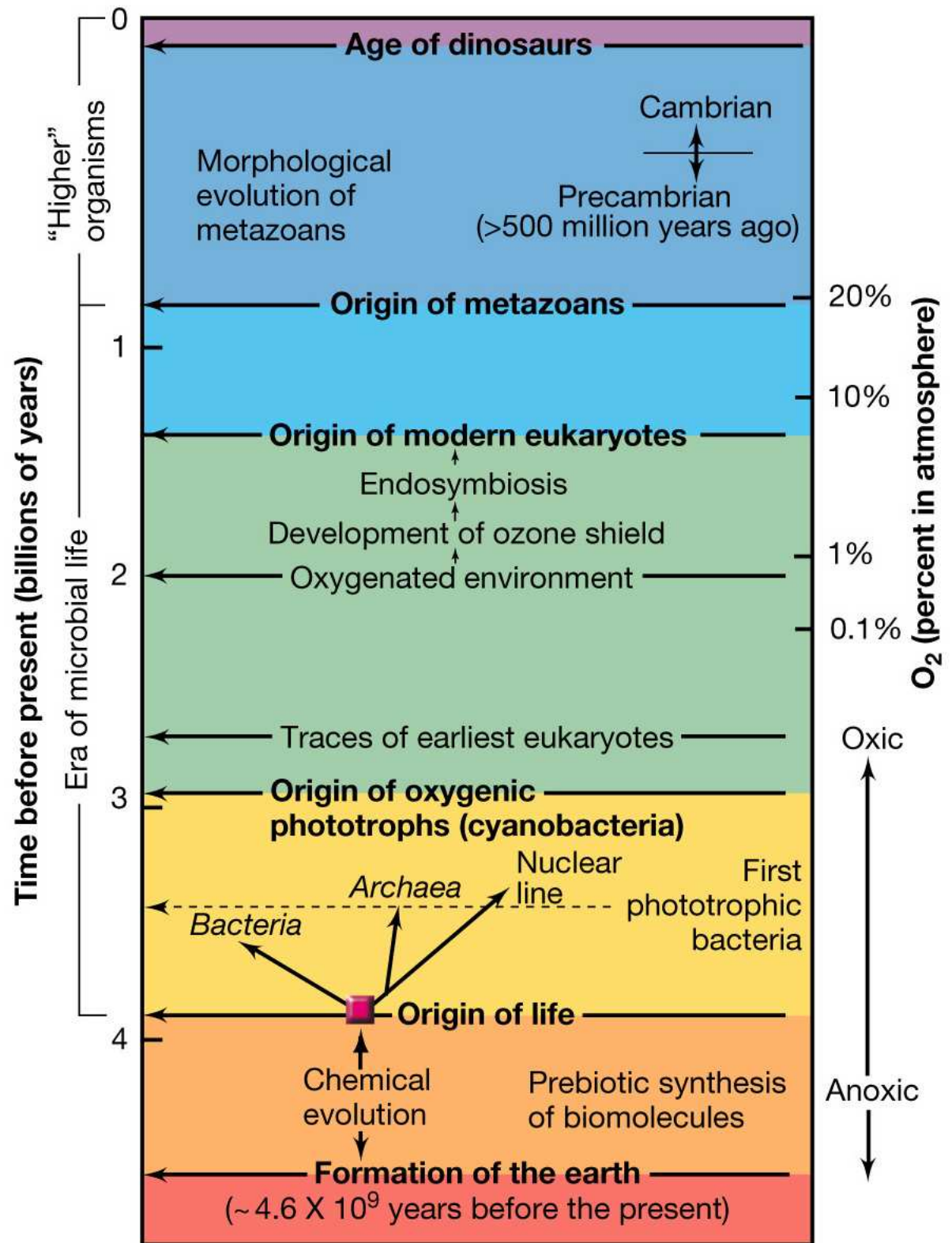
Livello	Batteri	Archea	Totale
Domini	1	1	2
Phyla	25	4 ^a	29
Classi	34	9	43
Ordini	78	13	91
Famiglie	230	23	243
Generi	1227	79	1306
Specie	6740	289	7029

^a I numeri rappresentano generi e specie di Batteri e Archea con nomi validi al 2005. La categoria Phyla per gli Archea include i Korarchaeota e i Nanoarchaeota, non ancora ufficialmente riconosciuti phyla.

Fonte: Garrity, G.M., Libum, T.G. e Bell, J.A., *Bergey's Manual of Systematic Bacteriology*, 2a ed., vol. 2, parte A, pp. 159-220, Springer-Verlag, New York, 2005.

**Microbial evolution and
systematics:
primitive microorganisms**

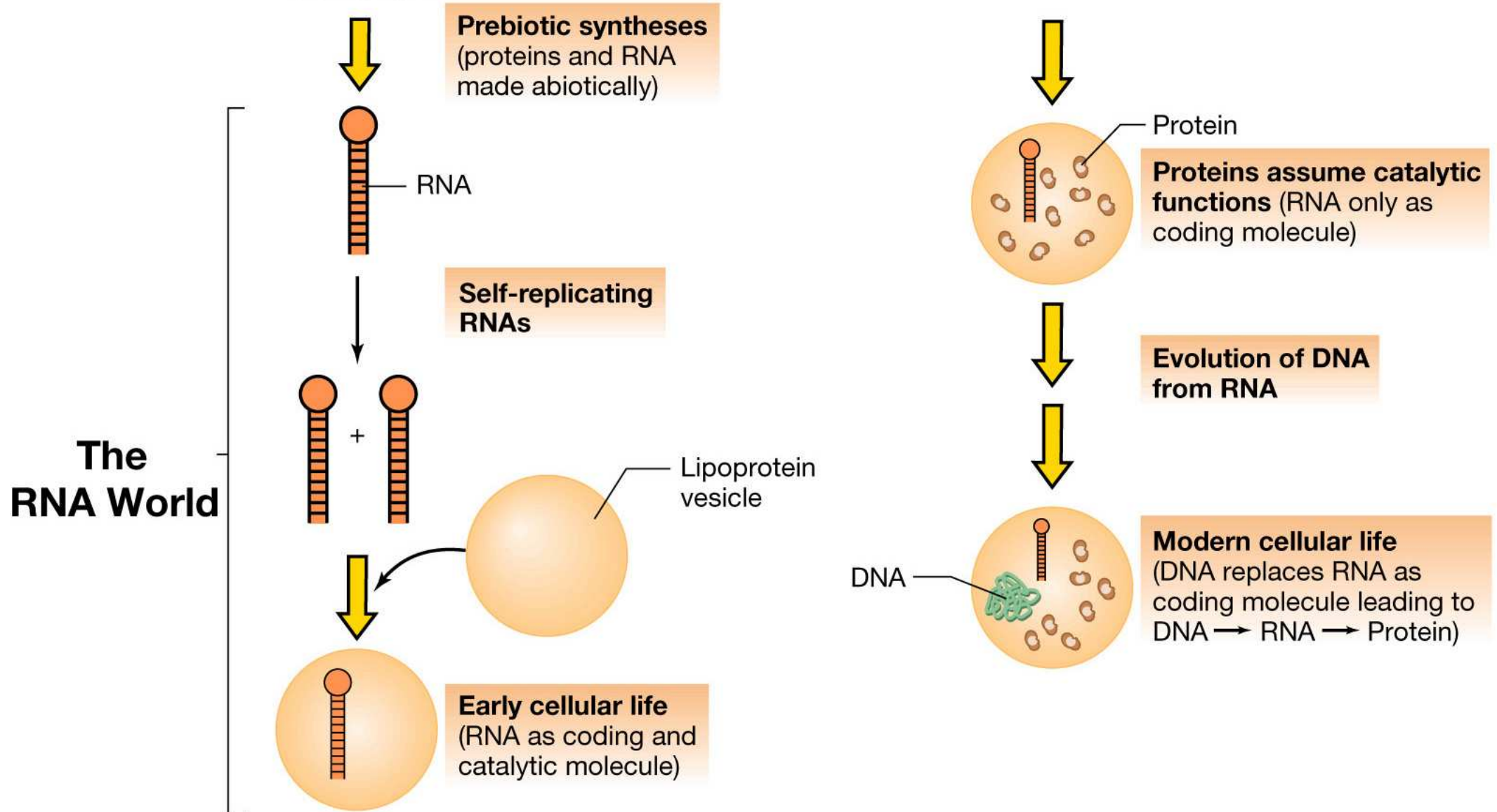
Major landmarks in biological evolution



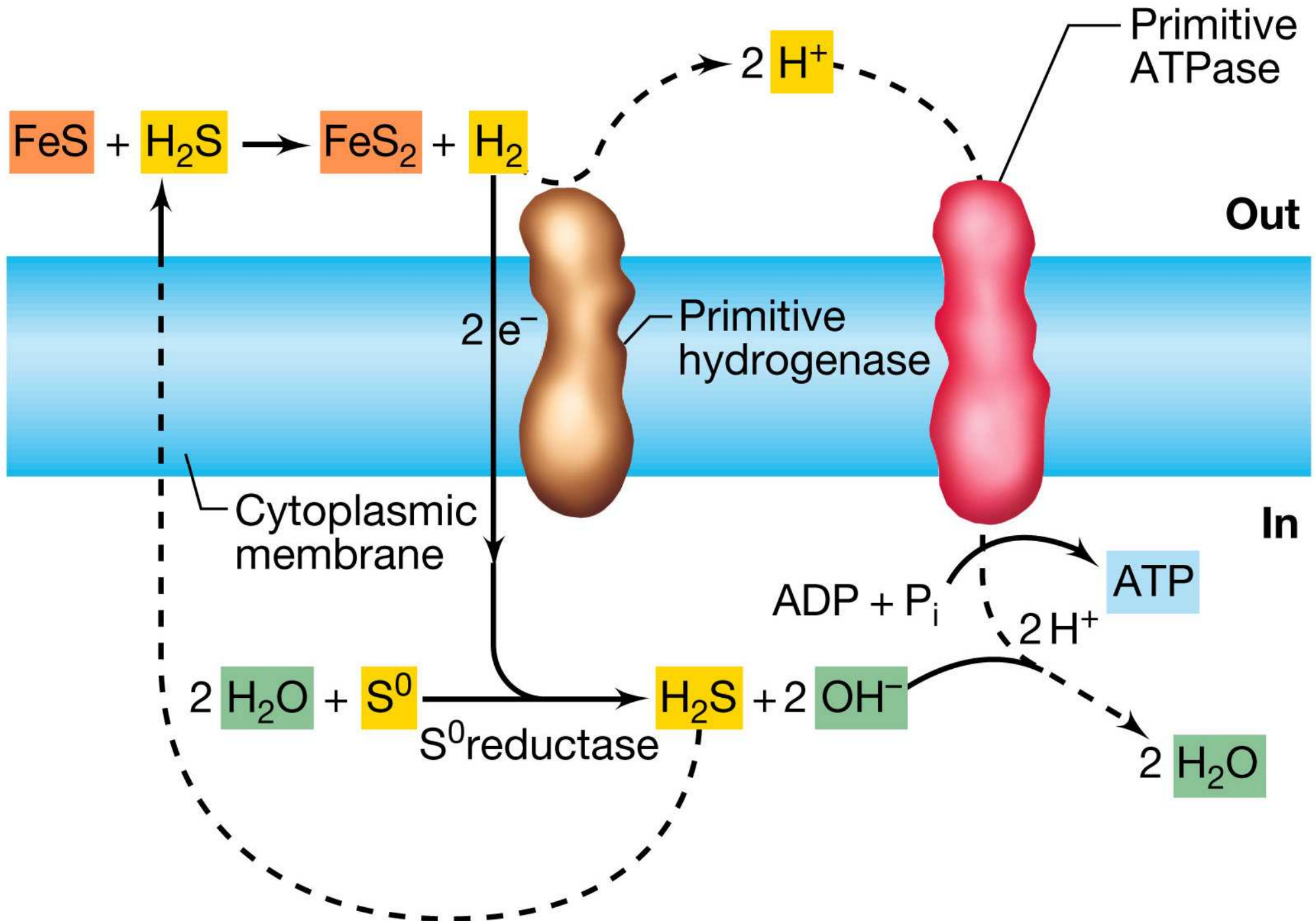
Possible scenario for the evolution of cellular life forms from RNA life forms



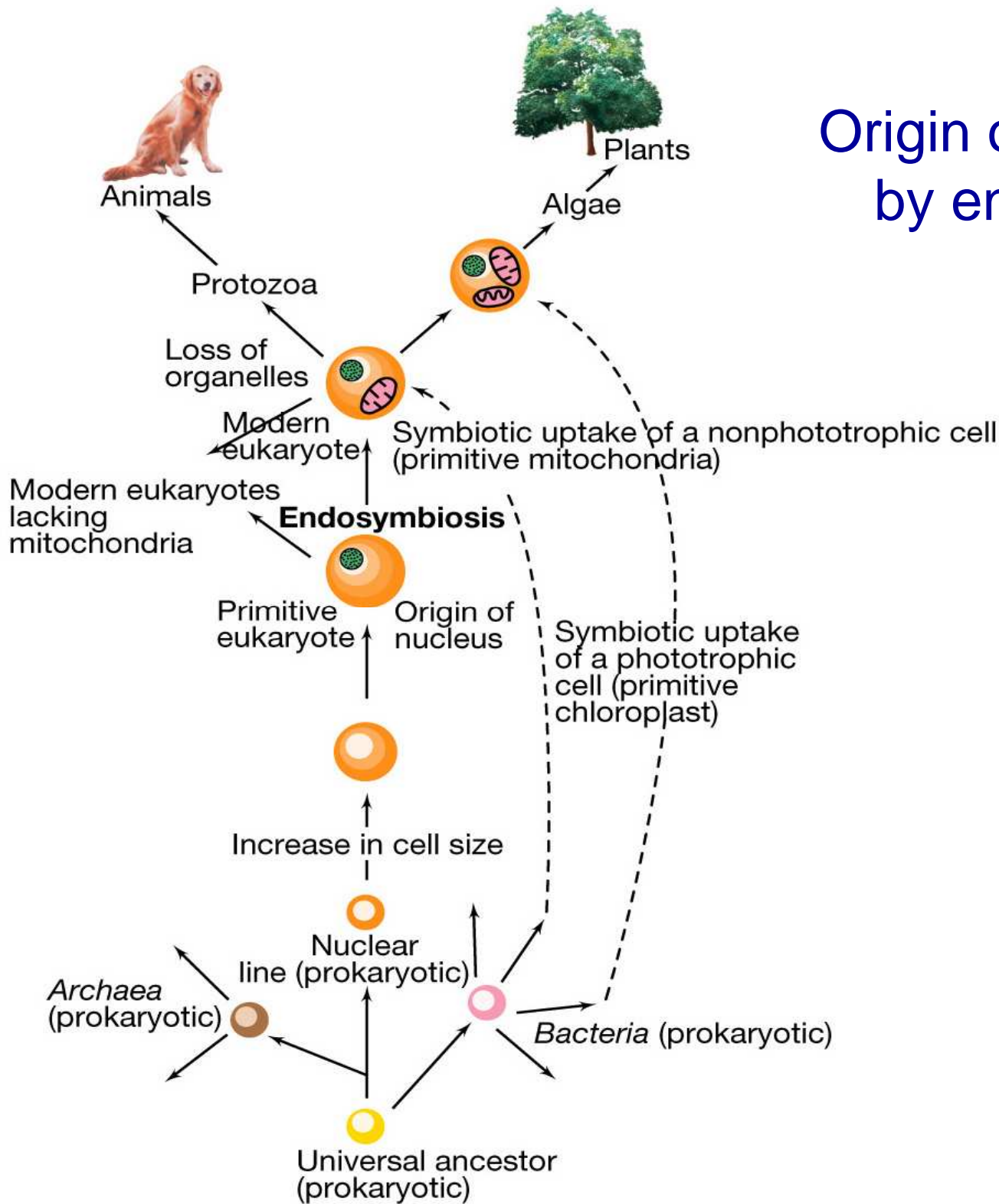
Sterile Earth



A hypothetical energy-generating scheme for primitive cells



Origin of modern eukaryotes by endosymbiotic events



Major landmarks in biological evolution

