

Endosymbiosis

Mitochondria.

- Origins.
- Energetic boost or burden.
- Functional remodeling.

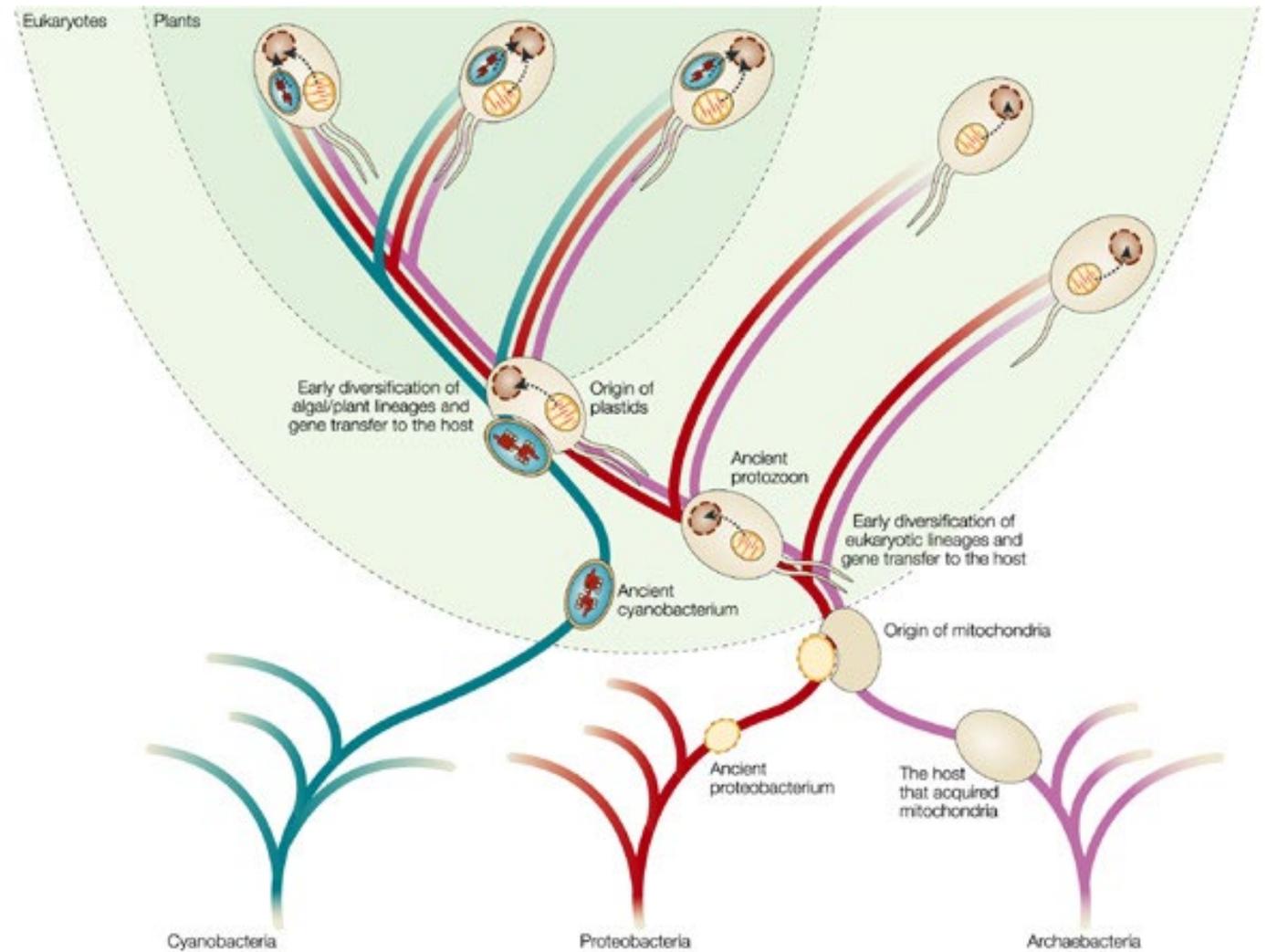
Extreme population-genetic environments.

- Mutation rates.
- Modes of inheritance.
- Muller's ratchet.

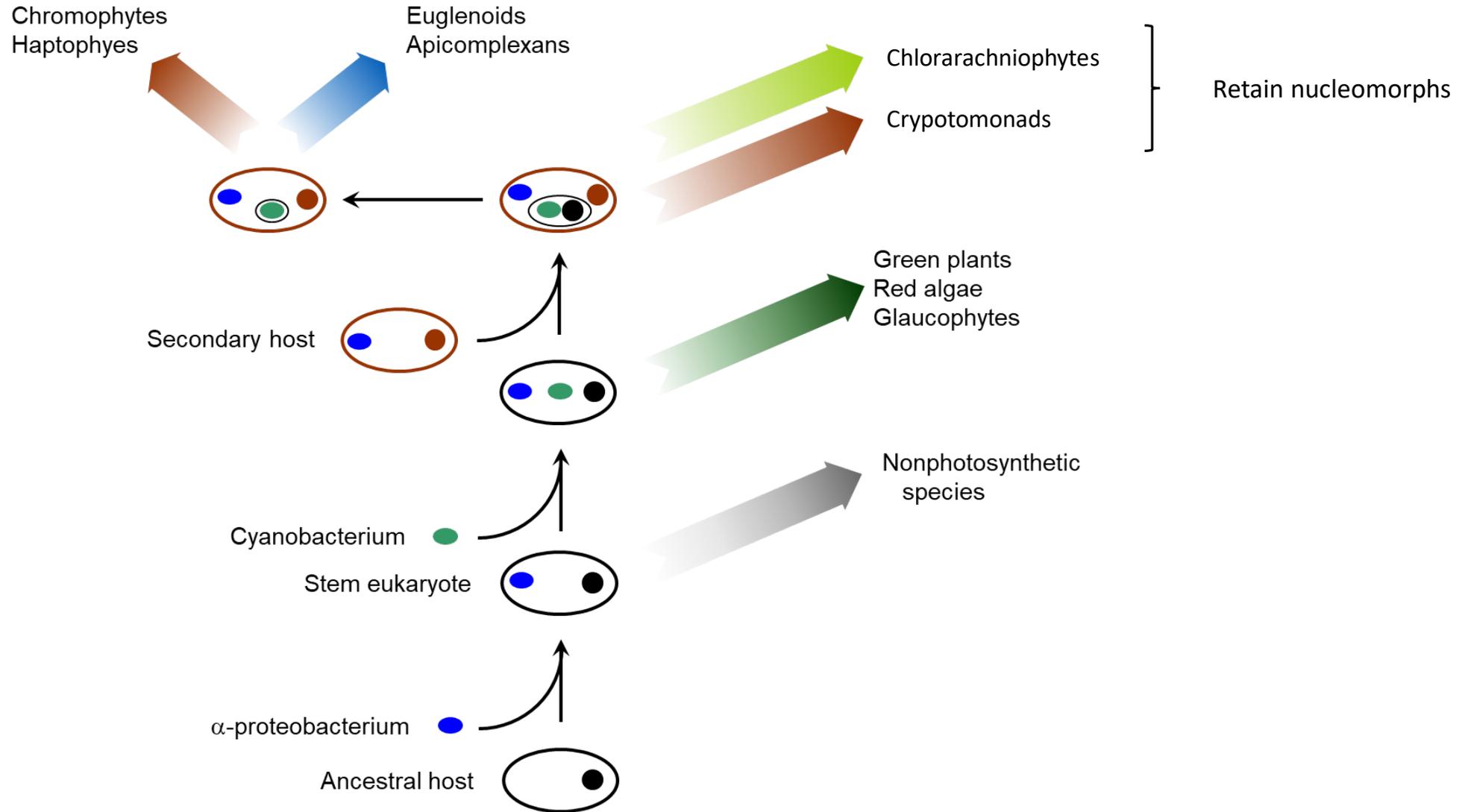
Organelle genome degradation.

- Animal mitochondrial tRNAs.
- Coevolutionary drive and compensatory mutations.

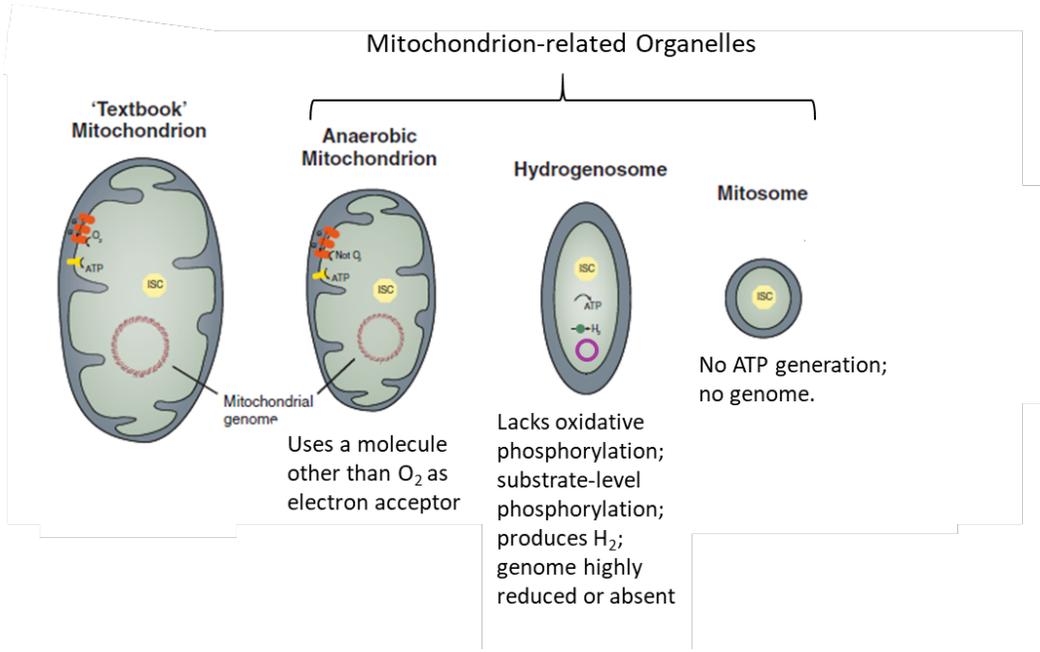
Addiction to endosymbionts.



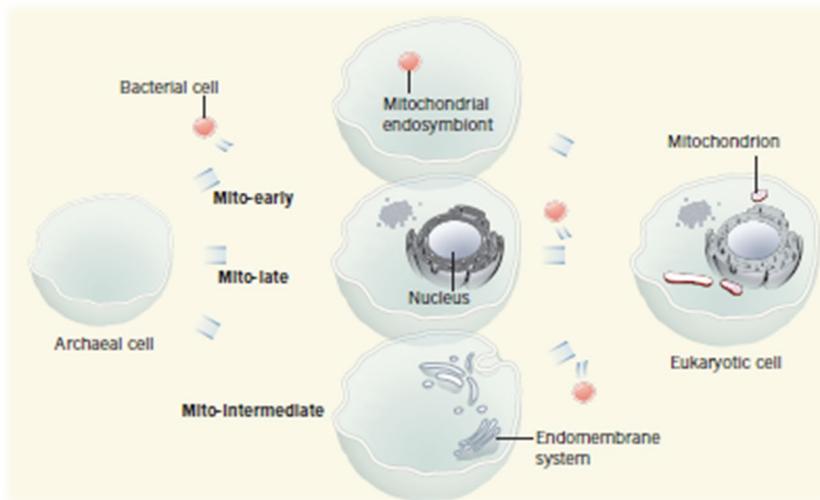
Downstream Secondary and Tertiary Endosymbiotic Events Involving the Chloroplast



Origin of the Mitochondrion: Open Questions



- From what microbial lineage did the mitochondrion emerge, and what might this tell us about the nature of the initial colonizer?
- From what microbial lineage was the host cell derived – bacterial, archaeal, or eukaryotic, and is the eukaryotic nucleus a descendant of that cell?
- Did the mitochondrion evolve after the establishment of the many other eukaryotic-specific attributes, or did it come first, with its presence somehow facilitating the origin of the latter?
- What, if anything, did the original host cell gain from the presence of its colonist and vice versa?



Mitochondria Contain Genomes, Usually Circular Like Bacteria

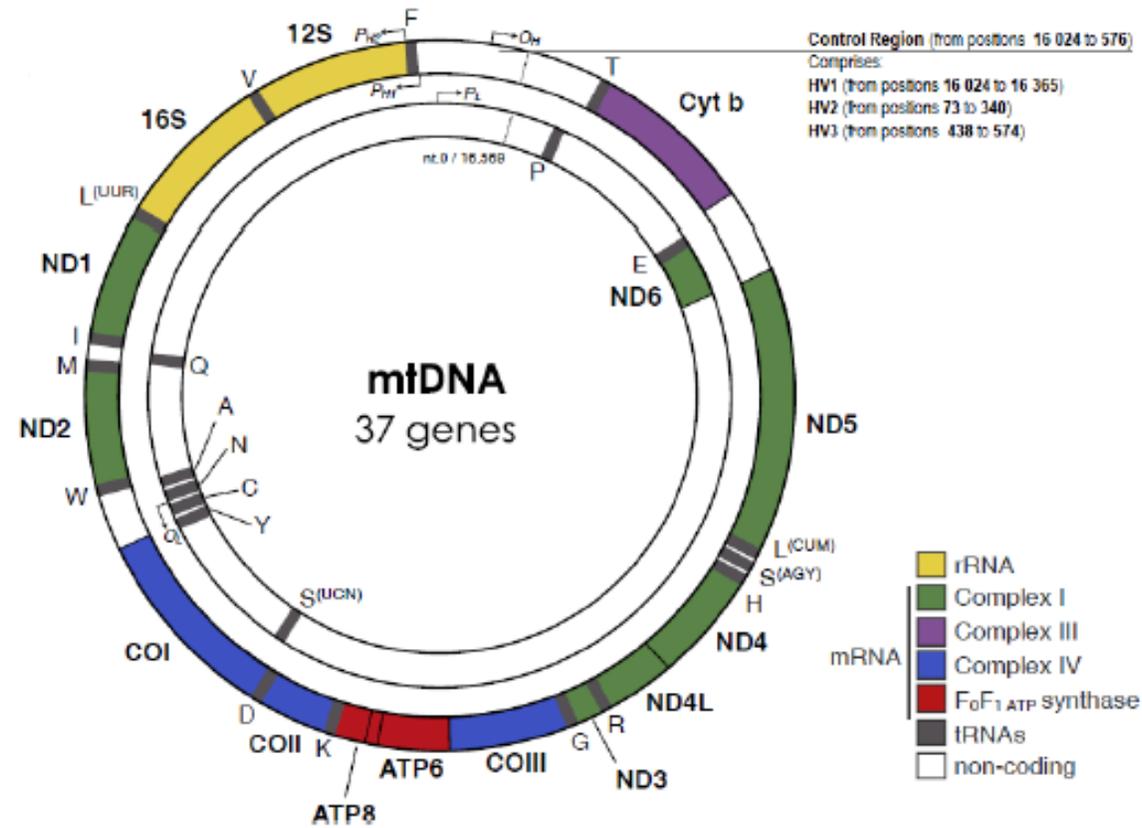
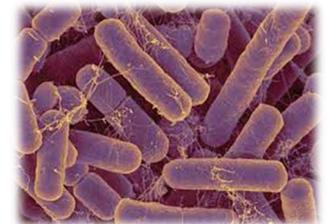
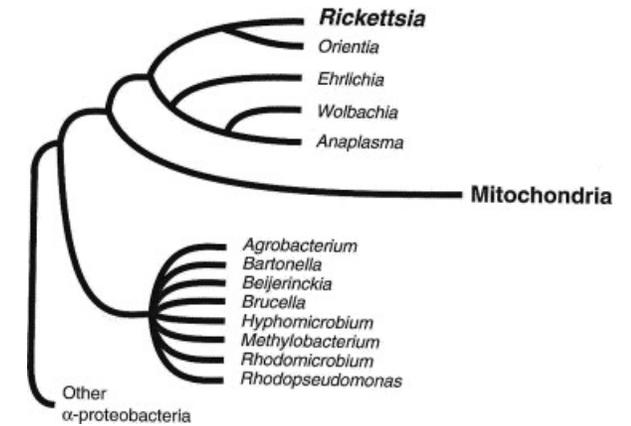


Figure 1 The human mitochondrial DNA genome with genes and control regions labeled. Adapted from *Picard, Wallace & Burrelle (2016)*.

Full-size  DOI: 10.7717/peerj.7314/fig-1

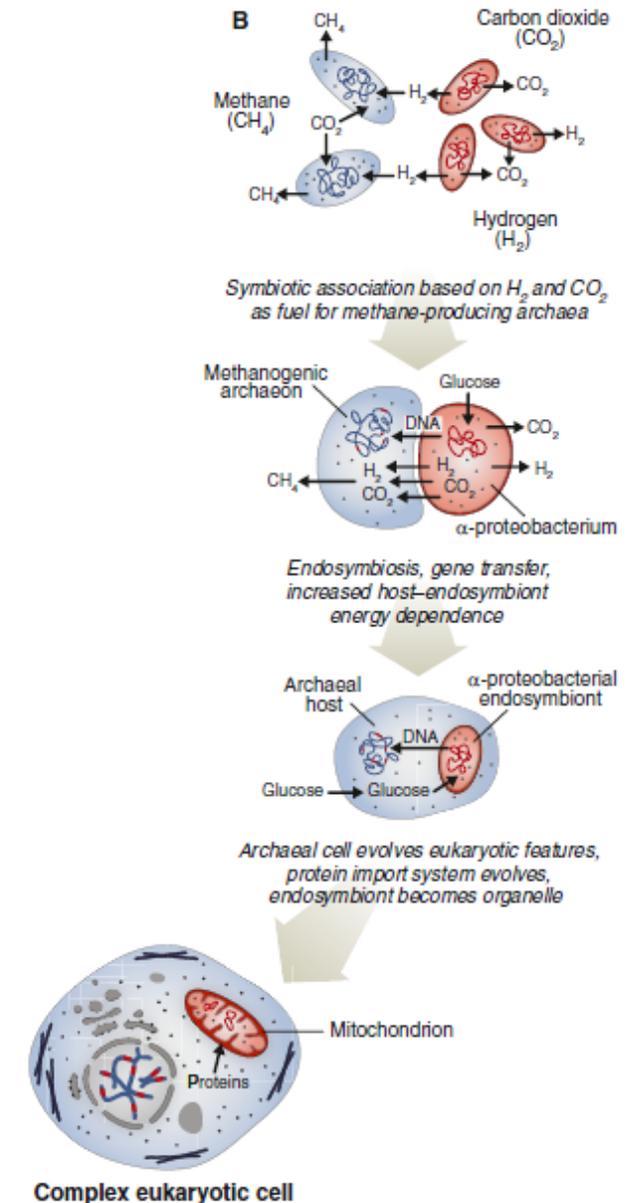
The Nature of the Ancestral Mitochondrion: which alpha proteobacterial group?

- One view: a derived member of the order Rickettsiales, all members of which (e.g., *Rickettsia*, *Wolbachia*, *Anaplasma*, *Orientia*) are intracellular parasites of eukaryotic cells (Andersson et al. 1998; Emelyanov 2001).
 - If this hypothesis is correct:
 - The primordial mitochondrion was an energy parasite, contrary to the common assertion that the mitochondrion gave a major energetic boost to its host.
 - LECA's mitochondrion initially harbored ~1200 genes; contained a transporter for ATP import from the host; had a flagellum; was capable of driving a TCA cycle, had an electron-transport chain allowing for oxidative phosphorylation; and carried out ribosomal biogenesis and fatty-acid synthesis.
- An alternative view: arose from a free-living anaerobic autotroph, although could still have been an energy parasite (Martin and Muller 1998; Cavalier-Smith 2006; Munoz-Gomez et al. 2017).
 - Invaginated membranes (cristae) and their junctions, upon which mitochondrial ETS complexes reside, appear to be homologous to intracellular membranes used in bioenergetic transactions by members of a large clade containing anaerobic photosynthesizers (purple nonsulfur bacteria), methanotrophs, and nitrite-oxidizing bacteria.



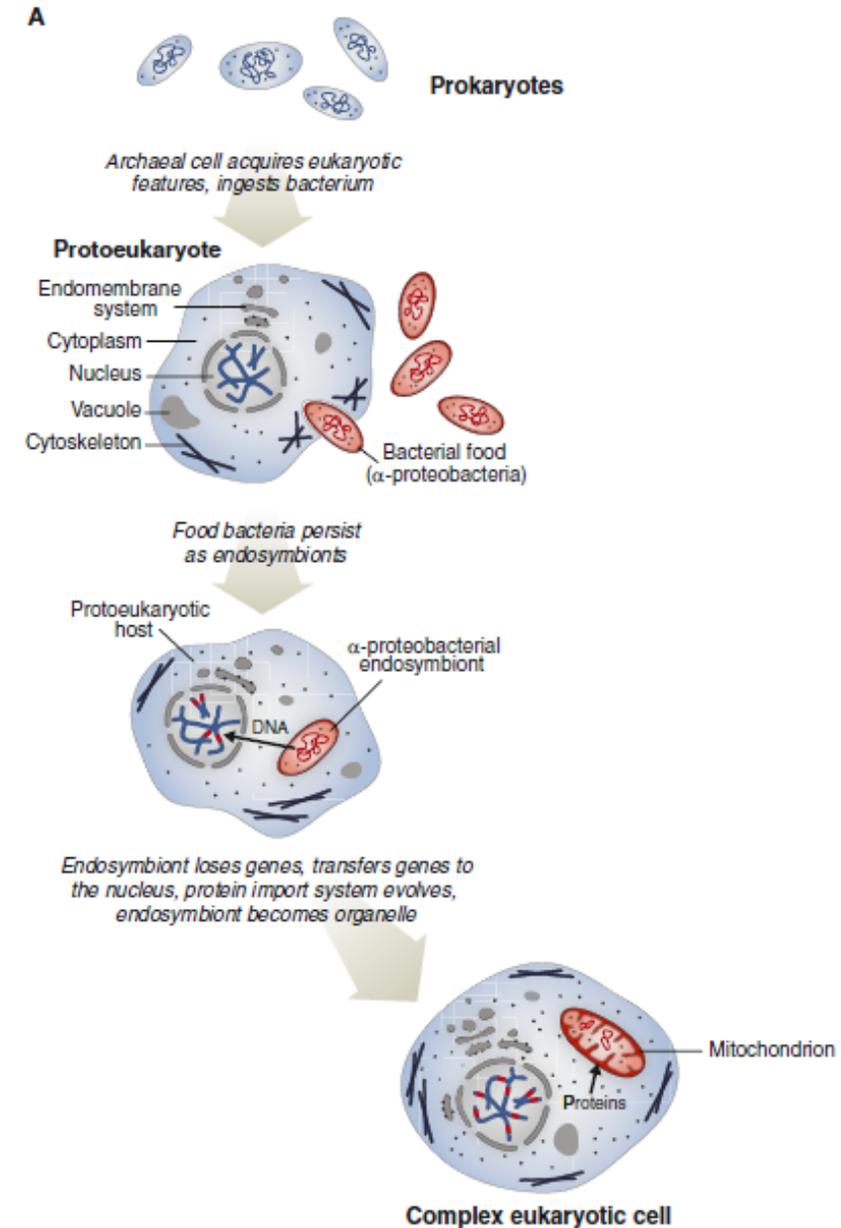
The Nature of the Ancestral Eukaryotic Host Cell

- Mitochondria-early views:
 - The hydrogen hypothesis – a methanogenic host cell consumed fuel (waste products) provided by a hydrogen-producing bacterium (Martin and Muller 1998).
 - The oxygen-scavenging hypothesis – the mitochondrion arose as a mechanism to remove toxic oxygen from an anaerobic host cell (Sagan 1967; Andersson et al. 2003).
- Assumes the host was a simple prokaryote with no internal membranes and no capacity for phagocytosis.
- There are no known cases of prokaryotes living inside of other free-living prokaryotes.



The Nature of the Ancestral Eukaryotic Host Cell

- Mitochondria-late view:
- Many of the early stages in eukaryogenesis, including the origin of a nucleus and internal membranes, were present prior to mitochondrial entry (de Duve 2007; Cavalier-Smith 2009; Pittis and Gabaldon 2016).



Adaptive Hypotheses for the Origin of the Mitochondrion:

the host or both partners acquire more resources than would be possible by living alone.

- Energy production – glycolysis in the cytoplasm converts glucose to pyruvate, which is then used in the mitochondrion to produce ATP.

But did the primordial mitochondrion have an ability and/or incentive to supply ATP to its host?

- Oxygen scavenging – rising O₂ levels ~2 BYA (the time of origin of eukaryotes) may have been toxic to primitive anaerobic eukaryotes.

- The Martin-Muller hypothesis – an original methanogenic anaerobe acquired hydrogen, a waste product, from the primordial mitochondrion.

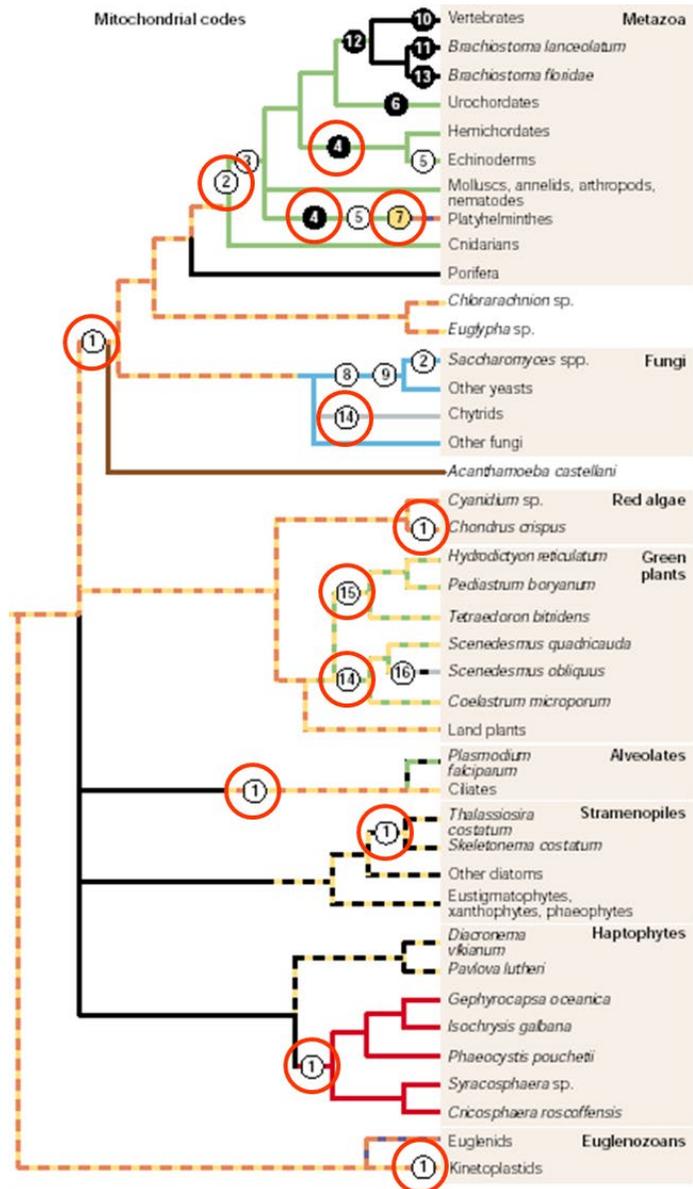
A few eukaryotes harbor a hydrogenosome, which produces hydrogen, acetate, and a small amount of ATP from anaerobic oxidation of glucose.

Under this hypothesis, the mitochondrion and the hydrogenosome represent alternative evolutionary pathways in which anaerobic vs. aerobic metabolism has been relinquished.

Stabilization of the Consortium by Nonadaptive processes: grand example of genome-wide subfunctionalization.

- How might evolutionary stability been achieved, particularly if one member of the original consortium was a parasite?
- Despite its disadvantages to the host, such a system would have been rendered stable if the host lost a key function that was complemented by the presence of the endosymbiont, and vice versa.
- Transfer of initially duplicated gene functions.
 - The mitochondrion forfeited nearly all genes for biosynthesis, replication, and maintenance to the nuclear genome.
 - The host cell abandoned key metabolic functions, such as membrane bioenergetics and iron-sulfur cluster biosynthesis, to the endosymbiont.
- Complete transfer has been thwarted in lineages that experienced genetic-code changes in the mitochondrion.

Genetic-Code Changes Are Common in Mitochondrial Genomes

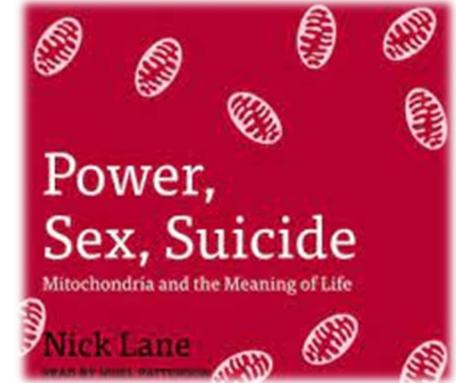


Many reassignments of stop codons and Reversions

- | | |
|------------------|------------------|
| ① UGA Stop → Trp | ⑤ AAA Lys → Asn |
| ② AUA Ile → Met | ⑥ AGR Ser → Gly |
| ③ AGR Arg → Ser | ⑦ UAA Stop → Tyr |
| ④ AUA Met → Ile | ⑧ CUN Leu → Thr |
| ⑨ CGN Arg → ? | ⑬ AGA ? → Ser |
| ⑩ AGR ? → Stop | ⑭ UAG Stop → Leu |
| ⑪ AGA ? → Gly | ⑮ UAG Stop → Ala |
| ⑫ AGR Ser → ? | ⑯ UCA Ser → Stop |

Energetic Boost or Burden?

- Maximum growth rates in bacterial species increase with cell size, whereas those in eukaryotes decline with cell size.
- The idea that complex internal structures are impossible in the absence of mitochondria is contradicted by the presence of intracytoplasmic membranes in several bacterial lineages.
- Eukaryotic species with reduced mitochondria or none at all still have elaborate internal and external complexities.
- The expansion of genome size in eukaryotes, thought by some to be essential to eukaryogenesis (Lane and Martin 2010), is readily explained by the increased power of random genetic drift in such lineages.
- ***A questionable premise:*** that an increase in energy availability provides evolution the freedom to do more evolutionary tinkering, driving diversification and a natural progression towards complexity.



Extraordinary Shifts in the Population-Genetic Environments of Mitochondria

- Increased mutation rates relative to those in nuclear genomes.
 - Humans ~2700x higher.
 - *Caenorhabditis* and *Daphnia* ~70x higher.
 - *Drosophila* ~10x higher.
 - Diatom ~2x higher.
- Uniparental inheritance.
 - Maternal to offspring transmission bottlenecks (only in multicellular species?)
 - Near complete absence of recombination.
- High mutation rates and lack of recombination lead to reductions in the efficiency of natural selection.

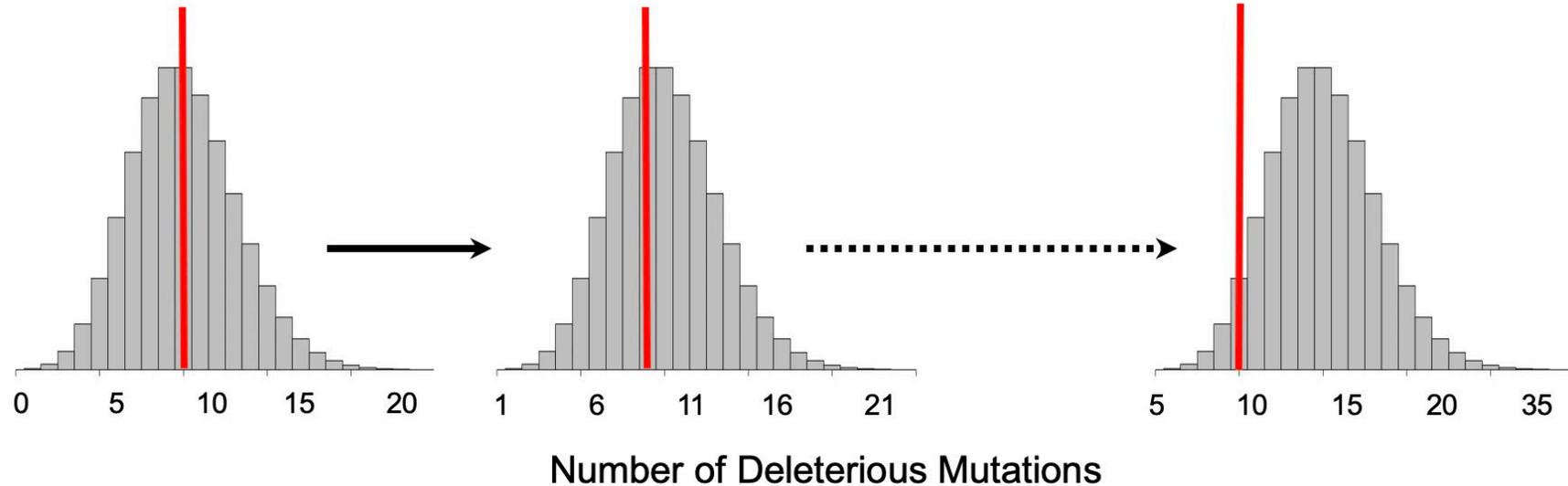
How Substantial is the Reduction in the Effective Population Size (N_e)?

- The classical “one-quarter” rule.
 - Two diploid nuclear genomes, but just a single maternally-derived mitochondrial genome, per mating.
 - Ignores issues associated with selective interference among linked mutations.

- Estimating relative N_e using joint information on standing variation at silent sites and known mutation rates:
 - The ratio of silent-site polymorphism in the same species (π_{sm} / π_{sn}) provides an estimate of $(2N_{gm}u_m / 2N_{gn}u_n)$.
 - Factoring out the ratio of mutation rates, u_m / u_n , yields an estimate of N_{gm} / N_{gn} .
 - For humans, $(\pi_{sm} / \pi_{sn}) \approx 5.5$, and $u_m / u_n \approx 2700 \rightarrow$ of $N_{gm} / N_{gn} \approx 0.002$.

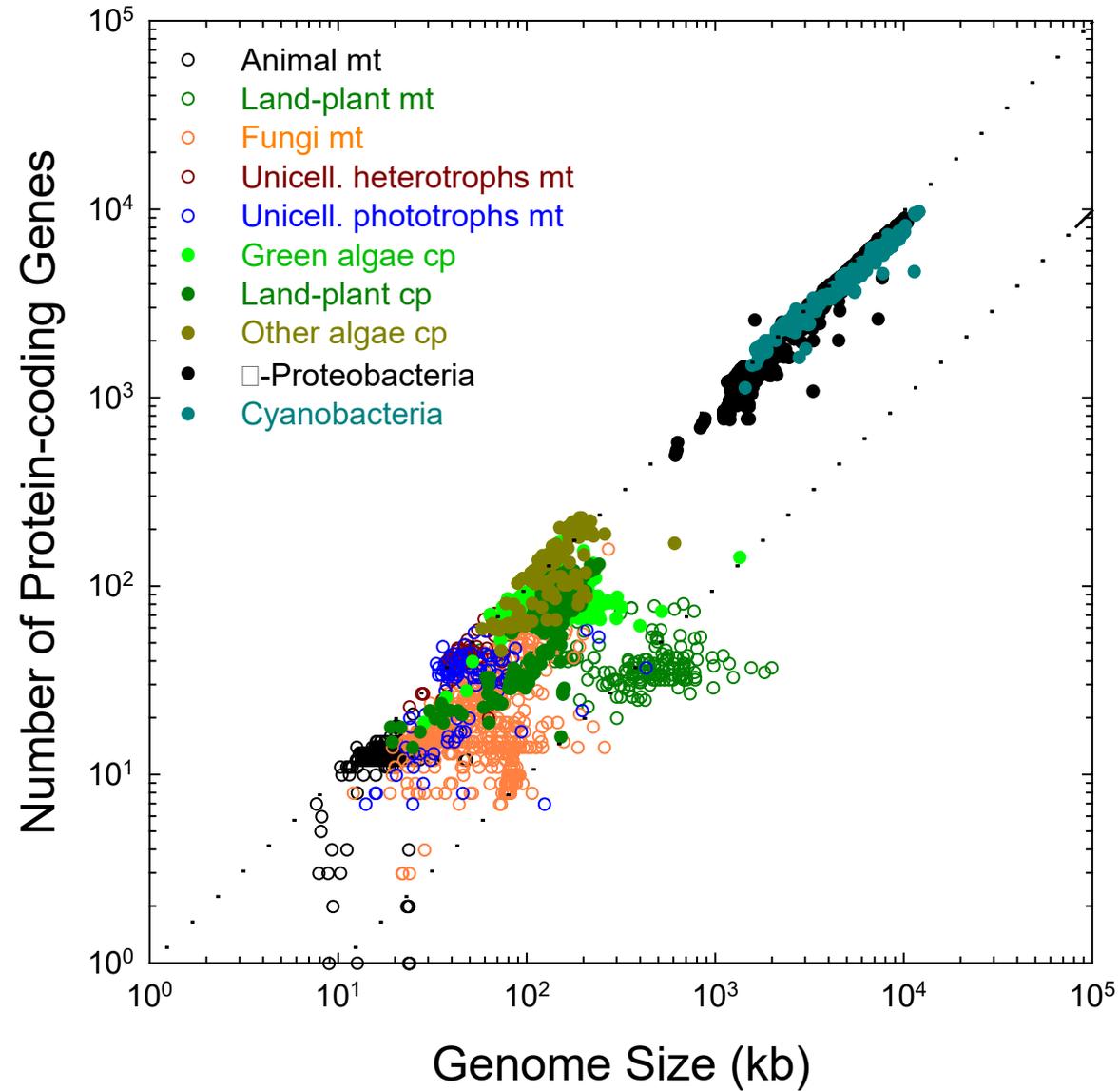
Mutational Meltdown Via Muller's Ratchet in Organelle Genomes?

Muller's Ratchet:



- Uniparental inheritance, combined with a high mutation rate and an absence of recombination, should greatly diminish the efficiency of selection, leading to a relentless accumulation of deleterious mutations.

Genome Miniaturization



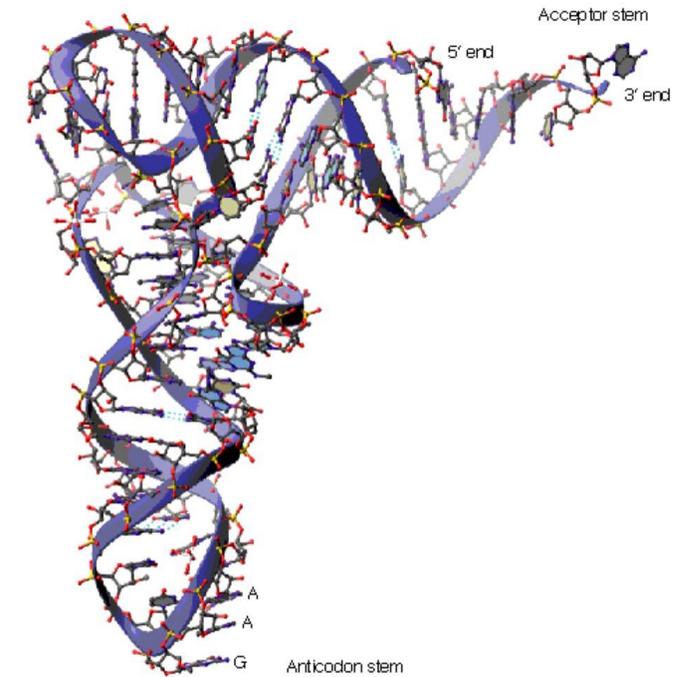
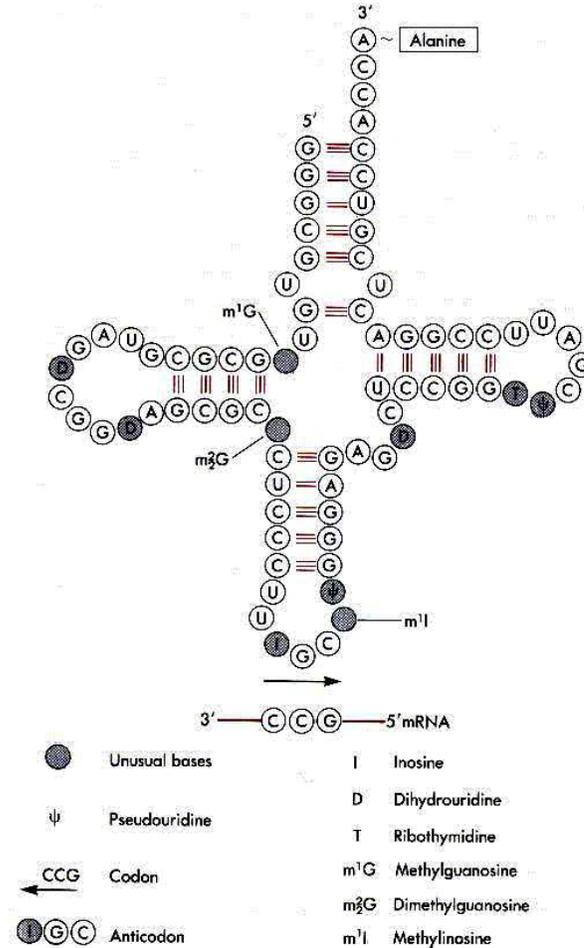
1 gene / kilobase

1 gene / 10 kilobase

Organelle Genome Degradation

- Comparison of mitochondrial- and nuclear-encoded genes with identical functions.

- 13 invariant tRNA bases across all bacteria and all nuclear genomes.



Transfer RNA

Loss of Conservation in tRNA Genes in the Mitochondrial Genomes of Animals

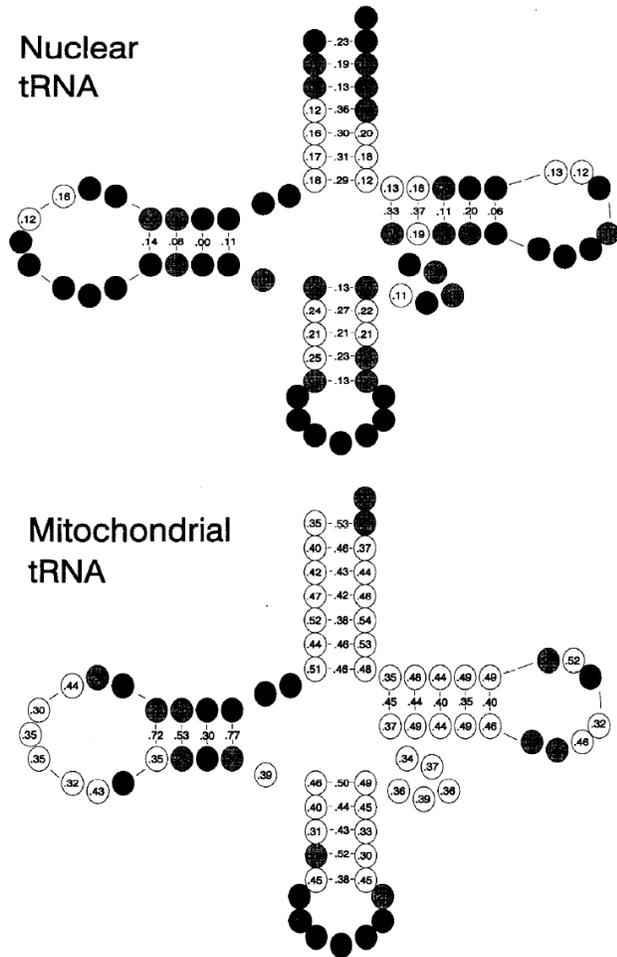


FIG. 2.—Estimates of average degrees of evolutionary lability for nucleotide sites (L_s) and stem pairs (L_p) in nuclear transfer RNA genes and for the homologous sites in mitochondrial genes. For the nuclear genes, sites with $0.0 \leq L_s \leq 0.05$ are shaded dark grey and those with $0.05 < L_s \leq 0.10$ are shaded light grey. For the mitochondrial genes, sites with $0.0 \leq L_s < 0.20$ are shaded dark grey and those with $0.20 \leq L_s \leq 0.30$ are shaded light grey. The thin lines in the D (left) and T (right) loops denote positions where insertions and deletions are common.

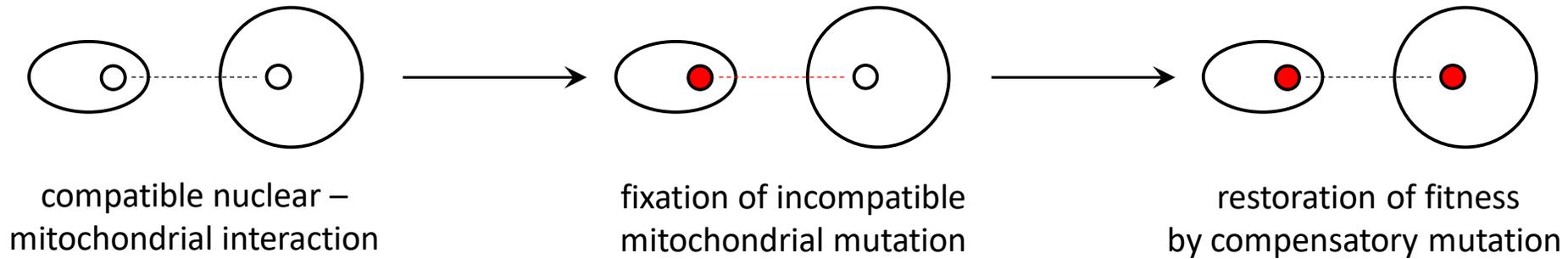
Table 3
Average Binding Strength of Stems (in Free Energy; $-\Delta G$ in kcal/mol, as Estimated at 37°C, 1 M NaCl)

	Mitochondrion	Nucleus
Acceptor	3.67 (0.29)	8.43 (0.29)
Dihydrouridine	1.13 (0.22)	2.98 (0.46)
Anticodon	2.23 (0.20)	4.80 (0.22)
T ψ C	2.22 (0.20)	5.27 (0.46)

Table 4
Mean Loop Sizes and Variation in Loop Size (Measured as the Average Absolute Proportional Deviation from the Mean)

	MEAN		VARIATION	
	Mitochondrion	Nucleus	Mitochondrion	Nucleus
Dihydrouridine	6.79 (0.23)	8.98 (0.25)	0.179 (0.013)	0.017 (0.008)
T ψ C	5.76 (0.15)	6.94 (0.15)	0.250 (0.023)	0.019 (0.018)
Variable	4.82 (0.10)	4.68 (0.12)	0.201 (0.019)	0.005 (0.003)
Anticodon	6.70 (0.16)	7.04 (0.04)	0.147 (0.017)	0.007 (0.005)

Cyto-Nuclear Coevolutionary Drive and Compensatory Mutations



Three types of nuclear-encoded genes make products that physically interact with products of organelle-encoded partners:

- 1) tRNA amino-acyl synthetases, each of which attaches a specific amino acid to its cognate tRNA, either in mitochondria or in cytosol;
- 2) ribosomal protein-coding genes designated for cytosolic vs. mitochondrial ribosomes;
- 3) nuclear-encoded components of the complexes in the mitochondrial oxidative phosphorylation (OXPHOS) pathway.

- Theory indicates that high mutation rates in organelles alone cannot drive positive selection in nuclear-encoded partners.
- The N_e of the organelle genome must also be small enough to drive the rate of *substitution* to levels beyond the neutral expectation (the mutation rate) in the nuclear genome.

Addiction to Endosymbionts: Beyond Subfunctionalization – Toxin-Antitoxin (TA) Systems

- Often carried on plasmids, but chromosomal TA systems are common.
- Thousands of systems are known – spread among bacterial species by horizontal gene transfer.
- Diversity of independently evolved toxicity mechanisms include transcription / translation inhibition, transcript destruction, interference with membranes or cell division.
- Toxins influence only their carriers.
- Antitoxin is less stable than the toxin.
- Cells that lose the TA complex die, as the toxin is released from inhibition.

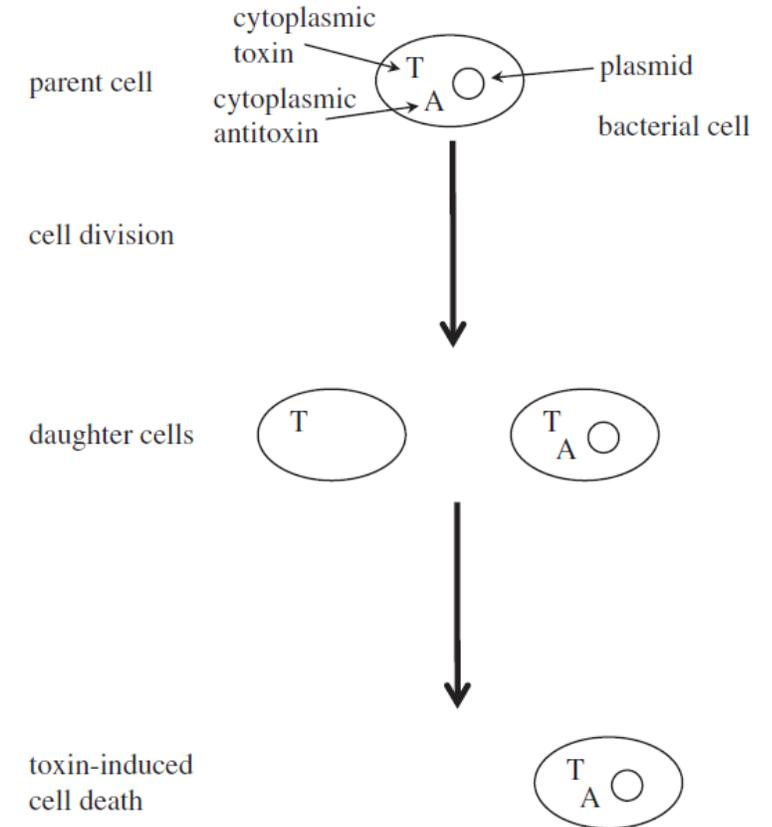
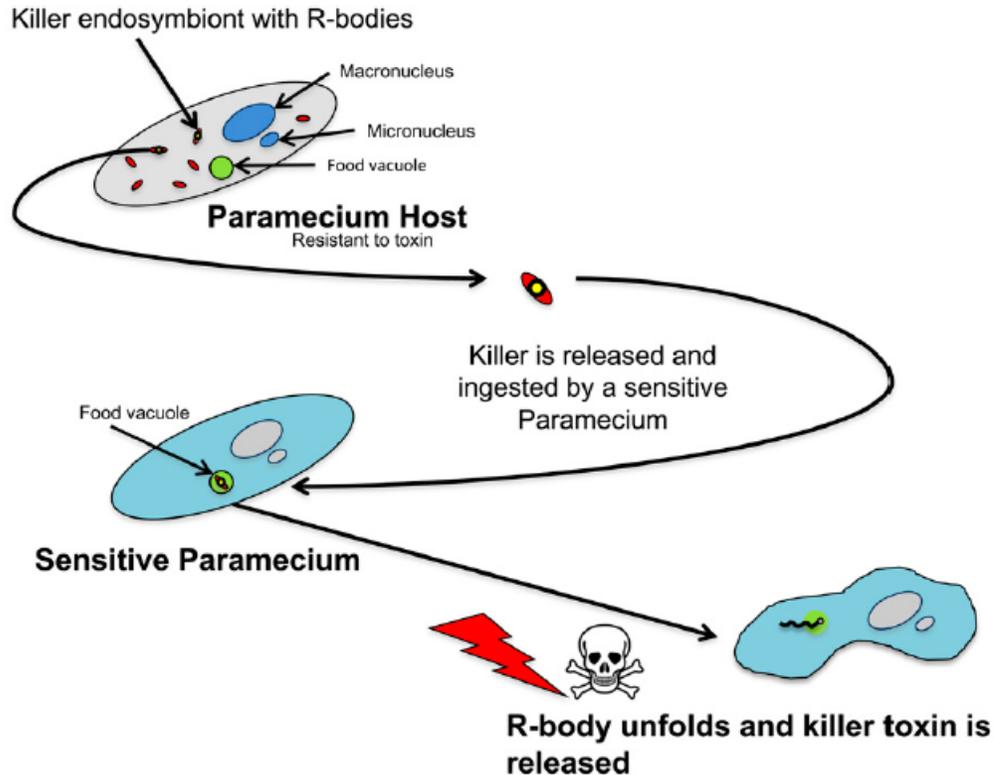


Figure 1. Schematic of plasmid addiction showing the presence (or the absence) of the toxin (T) and antitoxin (A) within the cytoplasm. If the plasmid is lost at cell division, then the antitoxin, which has a shorter half-life, quickly degrades and only the toxin is left. As a result, the daughter cell is killed.

The Killer Bacteria of *Paramecium*: Sonneborn's Kappa Particles



- R-body production is a terminal developmental stage.
- R bodies are necessary but not sufficient killing mechanisms.
- The mechanism of toxicity is unknown.
- The mechanism of host immunity is unknown.
- What the host and the occupant gain from the interaction is unknown.



Tracy Sonneborn
(1905 – 1981)

- Up to 50% of wild *Paramecium* isolates can carry killer “endosymbionts”.

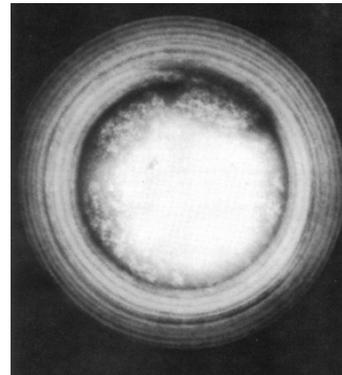
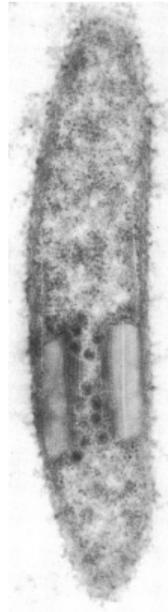
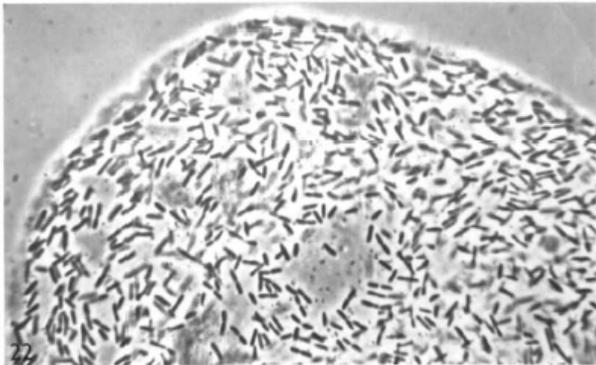
TABLE 2. Distinguishing characteristics of R-body-producing bacteria

Species	Host (site of endosymbiosis)	Extrachromosomal elements	R-body type	Killing
<i>C. taeniospiralis</i>	<i>P. tetraurelia</i> (cytoplasm)	Plasmid	51	Hump killing ^a
<i>C. varicaedens</i>	<i>P. biaurelia</i> (cytoplasm)	Phage	7	Various types
<i>C. pseudomutans</i>	<i>P. tetraurelia</i> (cytoplasm)	Phage	7	Spin killing
<i>C. paraconjugatus</i>	<i>P. biaurelia</i> (cytoplasm)	Phage	7	Mate killing
<i>C. caryophila</i>	<i>P. caudatum</i> (macronucleus)	Phage	Cc	Paralysis ^b



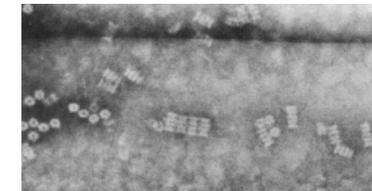
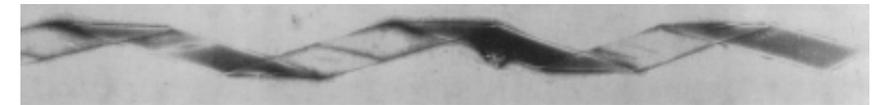
John Preer

Bacteria within *Paramecium* cell

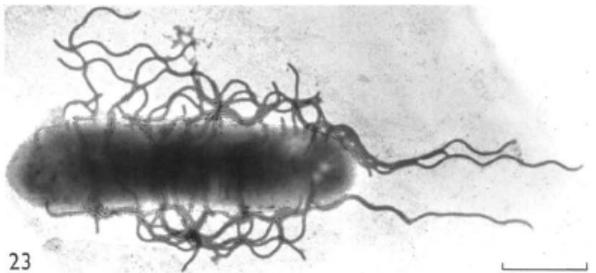


Cross section of R body

Unwound R body



Defective phage particles



Phylogenetically Diverse Killers

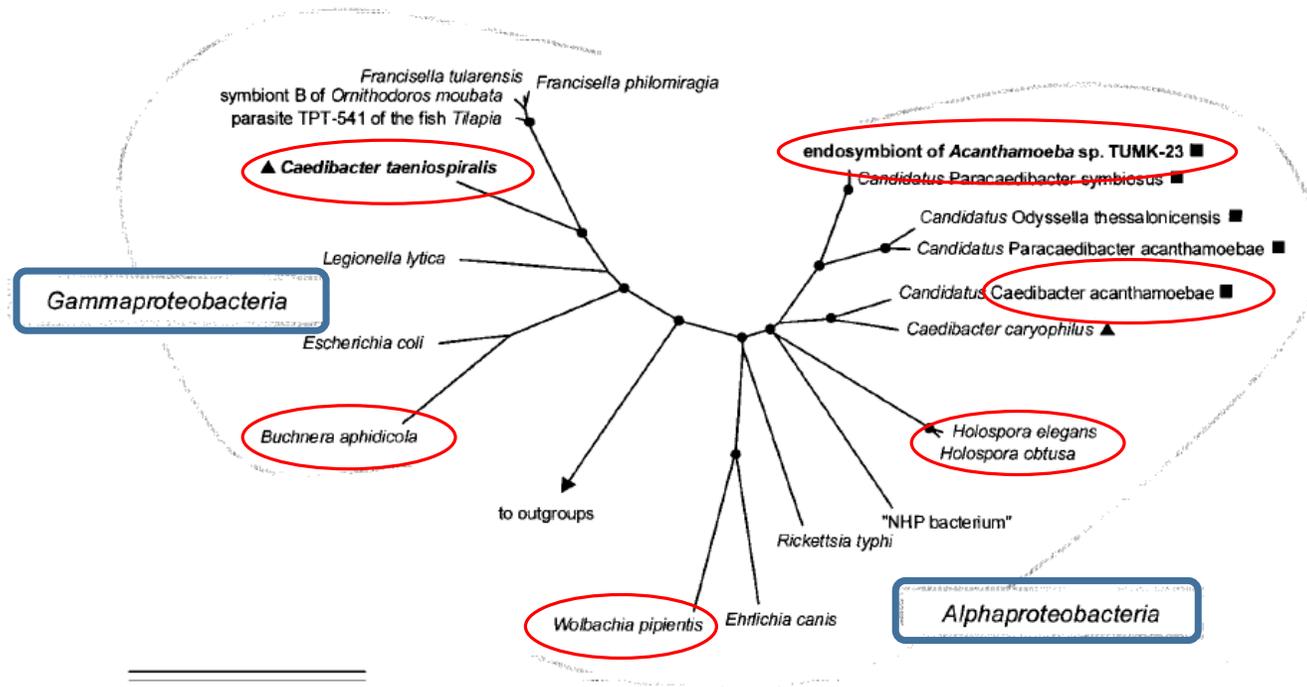


FIG. 3. 16S rRNA-based neighbor-joining tree showing (i) the phylogenetic affiliation of *C. taeniospiralis* 51k (endosymbiont of *P. tetraurelia*) with representative members of the *Gammaproteobacteria* and (ii) the relationship of the endosymbiont of *Acanthamoeba* sp. strain TUMK-23 with the alphaproteobacterial *C. caryophilus* (endosymbiont of *P. caudatum*) and other representative members of the *Alphaproteobacteria*. "Candidatus *Caedibacter acanthamoebae*," "Candidatus *Paracaedibacter acanthamoebae*," "Candidatus *Paracaedibacter symbiosus*," and "Candidatus *Odysella thessalonicensis*" were recently described as endosymbionts of acanthamoebae (5, 18). The respective eukaryotic hosts are indicated by symbols: ▲, *Paramecium* sp.; ■, *Acanthamoeba* sp. Parsimony bootstrap values (1,000 resamplings) of >97% are indicated as solid circles. "NHP bacterium," shrimp pathogen causing necrotizing hepatopancreatitis. Bar, 10% estimated evolutionary distance.

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 0099-2240/02/\$04.00+0 DOI: 10.1128/AEM.68.12.6043-6050.2002
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Vol. 68, No. 12

The Genus *Caedibacter* Comprises Endosymbionts of *Paramecium* spp. Related to the *Rickettsiales* (*Alphaproteobacteria*) and to *Francisella tularensis* (*Gammaproteobacteria*)

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Received 23 May 2002/Accepted 2 September 2002

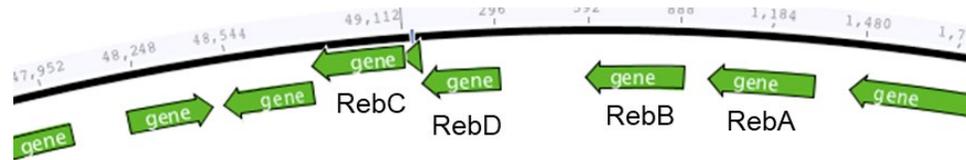
- The R body is formed by a set of duplicate RebA-D genes, each of which encodes for a small (70-110 AA) protein.
- Together, form higher-order polymers.
- Can be expressed in *E. coli*.

MICROBIOLOGICAL REVIEWS, Mar. 1989, p. 25-67
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Vol. 53, No. 1

R-Body-Producing Bacteria

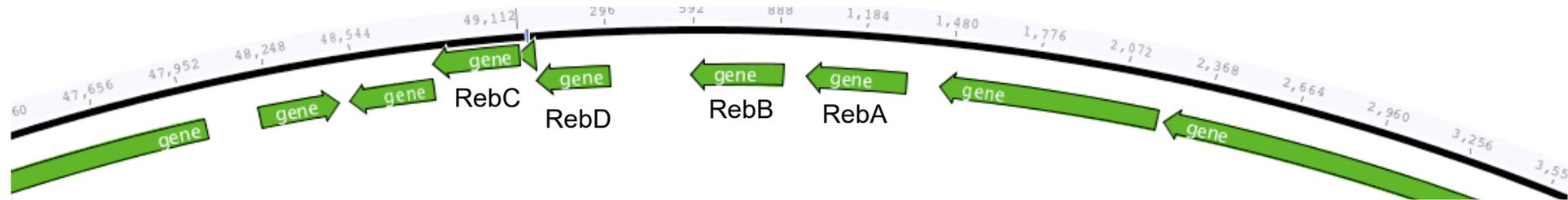
FINN R. POND,¹* IAN GIBSON,² JORGE LALUCAT,³ AND ROBERT L. QUACKENBUSH¹‡



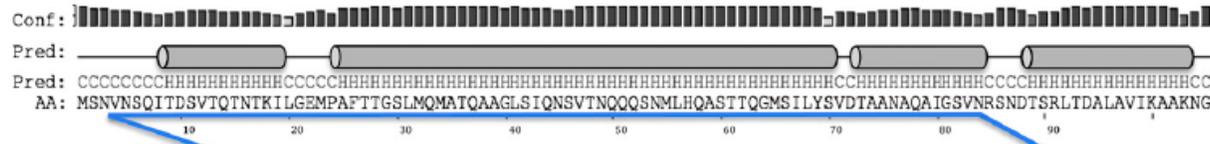
R body type	Ribbon dimensions		Ribbon Morphology			Mode of unrolling
	maximum length	width	Outer Terminus	Center	Inner Terminus	
51	20 μm	0.4 μm				
7	20 μm	0.4 μm				
Cc	$\geq 20 \mu\text{m}$	0.8 μm				
Pt	< 10 μm	0.25 μm				
Pa	30 μm	0.8 μm				

FIG. 1. Distinguishing features of different R-body types. The R bodies of strain EPS-5028 and *R. centenum* have not been described in detail and so are not included in any R body class. In the right-hand column, an arrow pointing down indicates unrolling from the inside outward. An arrow pointing to the side indicates unrolling from the outside. Note that the type Pa R body is drawn as a loose coil to better show its nature, although it is actually tightly coiled in its host cell. Included in the type 7 R-body class are the R bodies of *C. paracojugatus*, which are smaller than other type 7 R bodies (about 5 μm long and 0.2 μm wide).

- The R body is formed by the RebA-D genes, which are paralogs of each other.
- Reb genes code for small (70-110 AA) proteins.



Wide Distribution of the Reb Genes Across the Bacterial Phylogeny



B

