Systems Microbiology

Monday Oct 29 - Andersson and Moran readings

Genome Evolution & Ecology

- LGT & Genome Evolution
- Genomics of Endosymbionts
- Environmental Genomics

Mechanisms and consequences of Lateral Gene Transfer

Figure by MIT OCW.

Transduction:
via a virus (bacteriophage)

Conjugation:
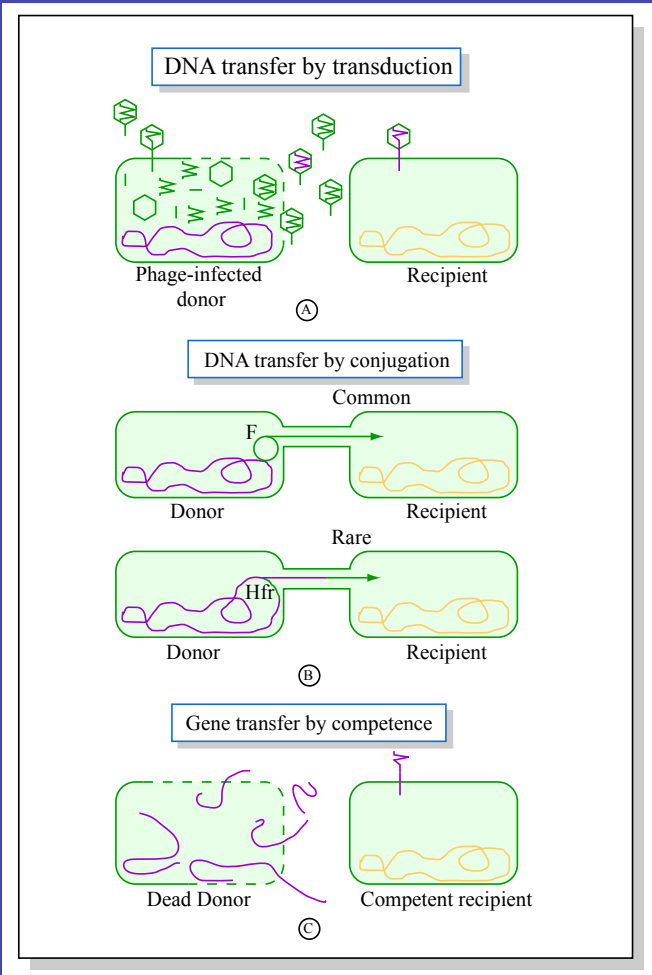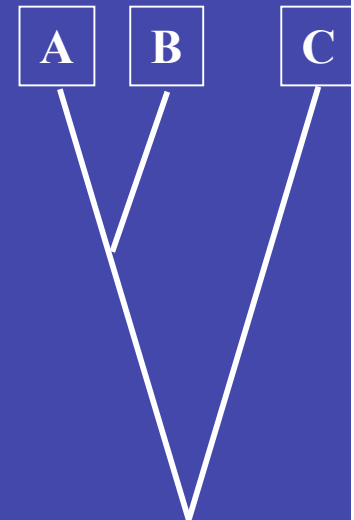direct contact (plasmid)

Transformation:
integration of free DNA

DNA transfer by transduction

Phage-infected donor | Recipient
Ⓐ

DNA transfer by conjugation

Common
F
Donor | Recipient

Rare
Hfr
Donor | Recipient
Ⓑ

Gene transfer by competence

Dead Donor | Competent recipient
Ⓒ

Organismal phylogeny

A B C

gene phylogeny

A B C

Microscopic photographs of phage and bacterial conjugation removed due to copyright restrictions.

POPULATION GENETICS OF PATHOGENS

MG1655 (K-12)
Non-Pathogenic

CFT073
Uropathogenic

193
2.5%

585
7.6%

1623
21.2%

2996
39.2%

514
6.7%

204
2.6%

1346
17.6%

EDL933 (O157:H7)
Enterohaemorrhagic

Total proteins = 7638
2996 (39.2%) in all 3
911 (11.9%) in 2 out of 3
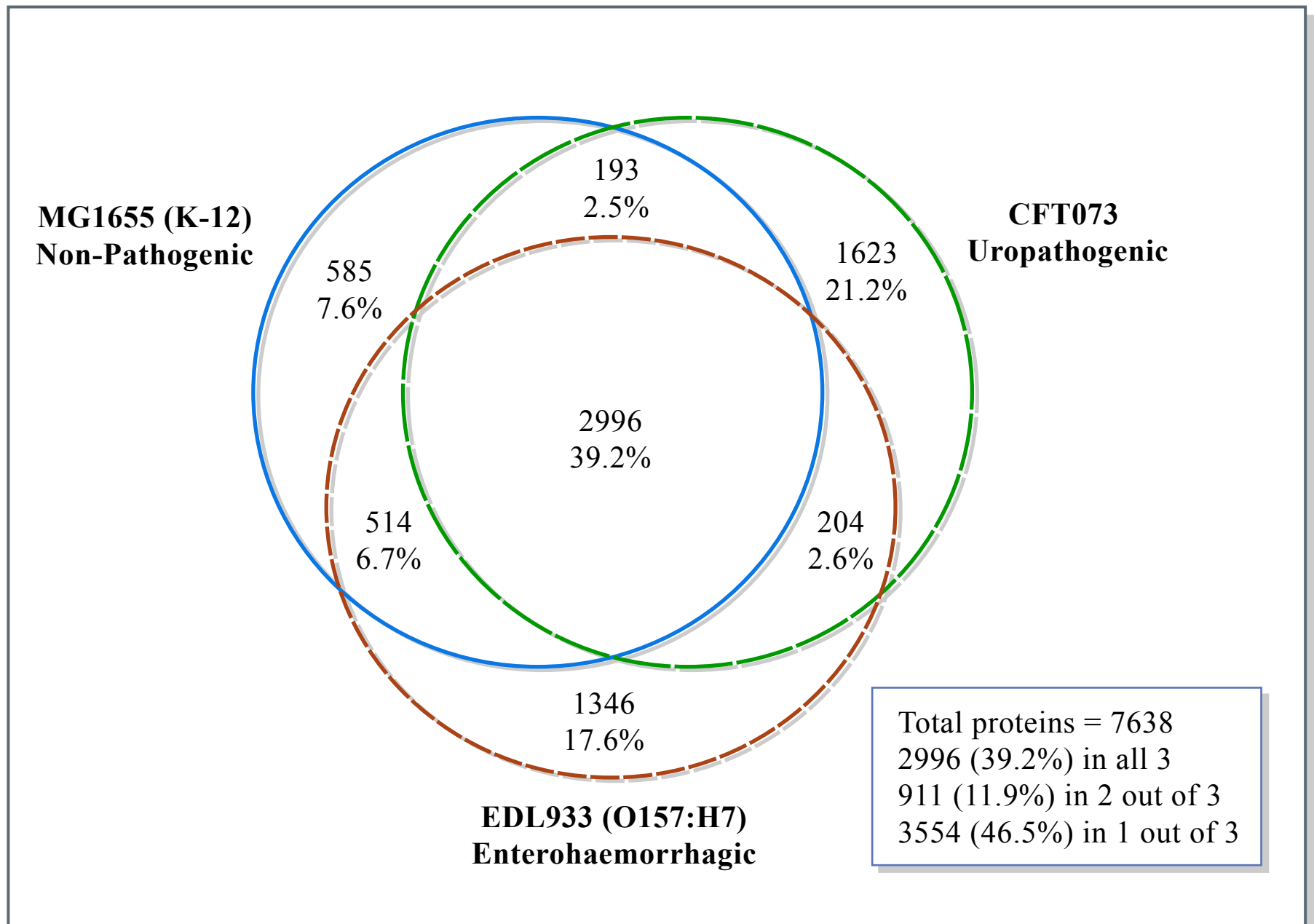3554 (46.5%) in 1 out of 3

Figure by MIT OCW.

UROPATHOGENIC & ENTEROHAEMORRHAGIC "HOT-SPOTS"

Welch, R. A. et al. (2002) Proc. Natl. Acad. Sci. USA 99, 17020-17024
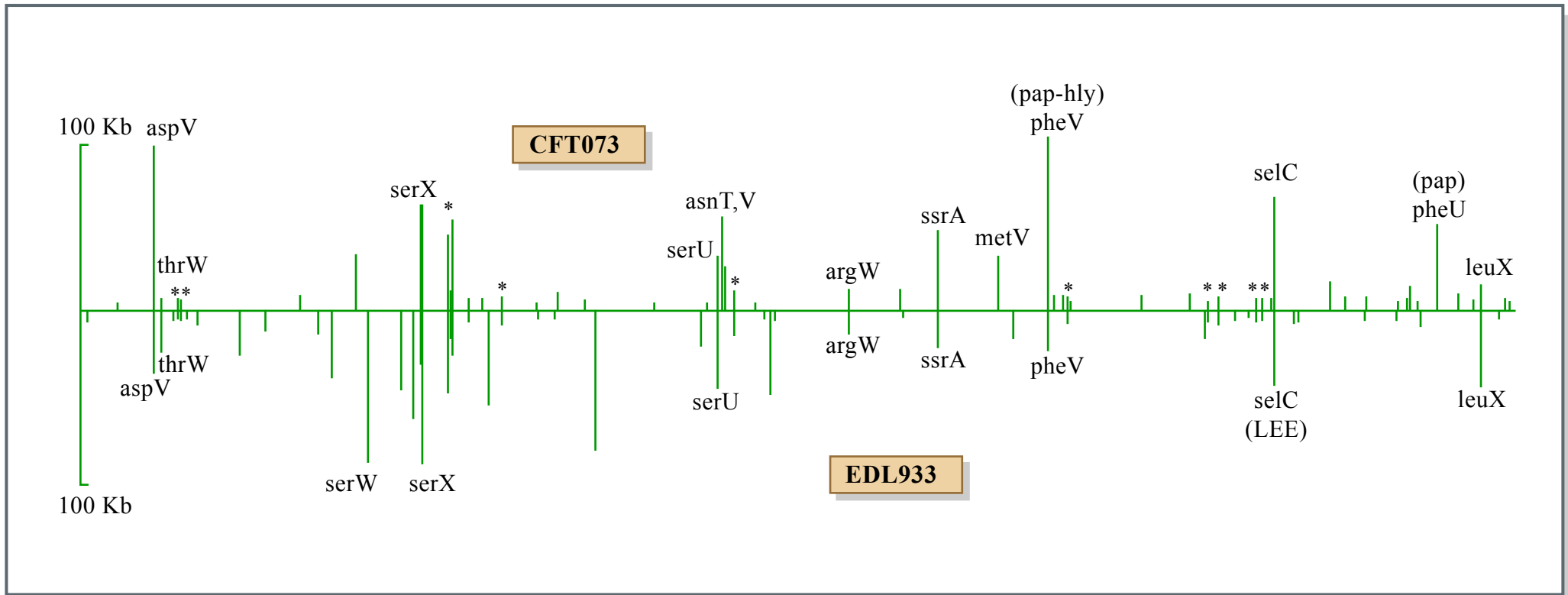
POPULATION GENETICS OF PATHOGENS

Figure by MIT OCW.

UROPATHOGENIC & ENTEROHAEMORRHAGIC "HOT-SPOTS"

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Molecular Phylogenetics :
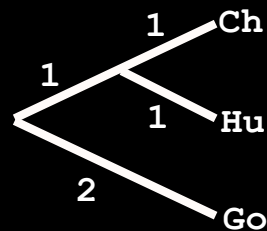## Inferring Evolutionary Relationships

Gorilla	ACGT**CGTA**	
Human	ACGTTCCT	1. Construct multiple alignment of sequences
Chimpanzee	ACGTT**T**C**G**	

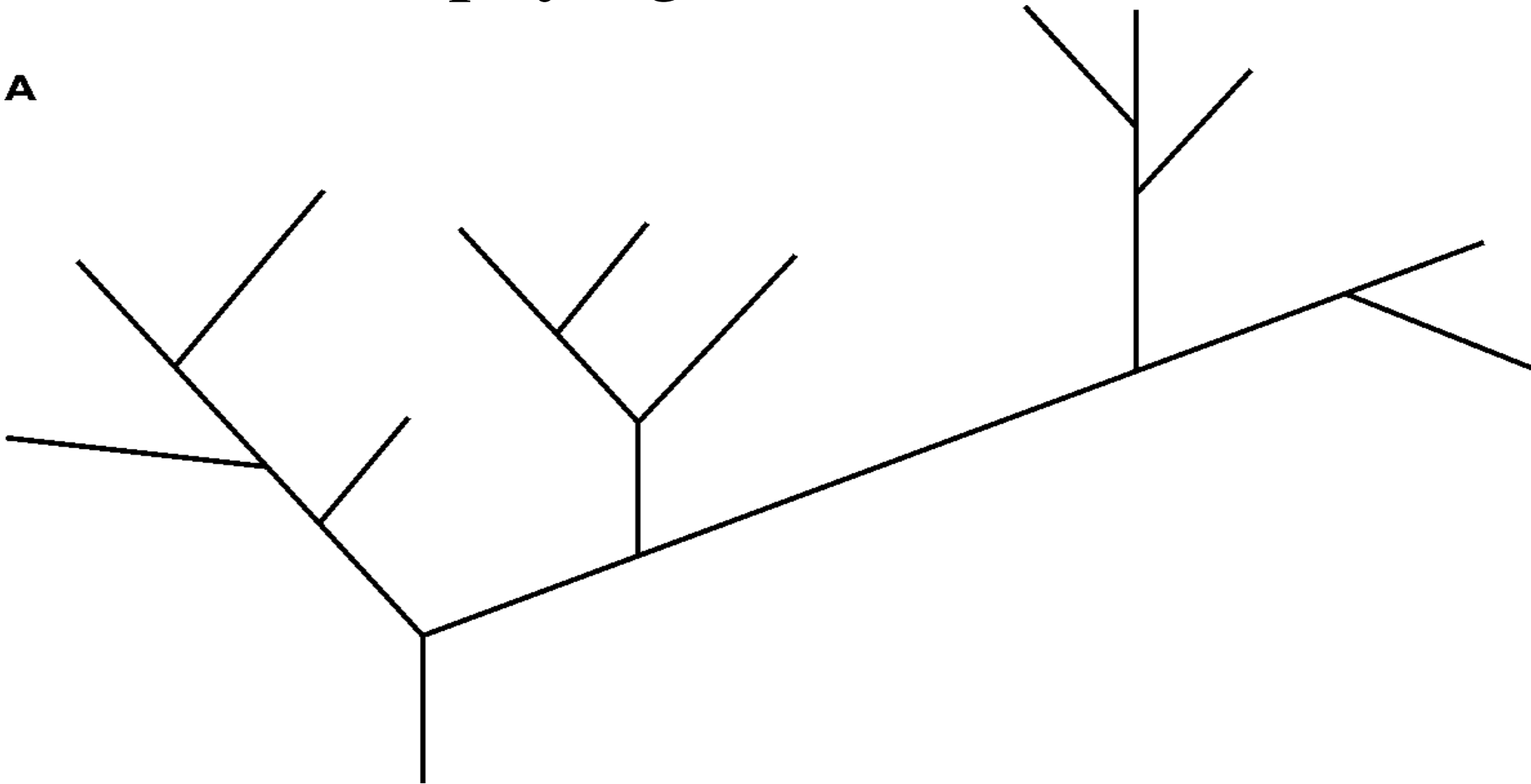|	Go	Hu	Ch
Go	–	4	4
Hu		–	2
Ch			–

2. Construct table listing all pairwise differences matrix)
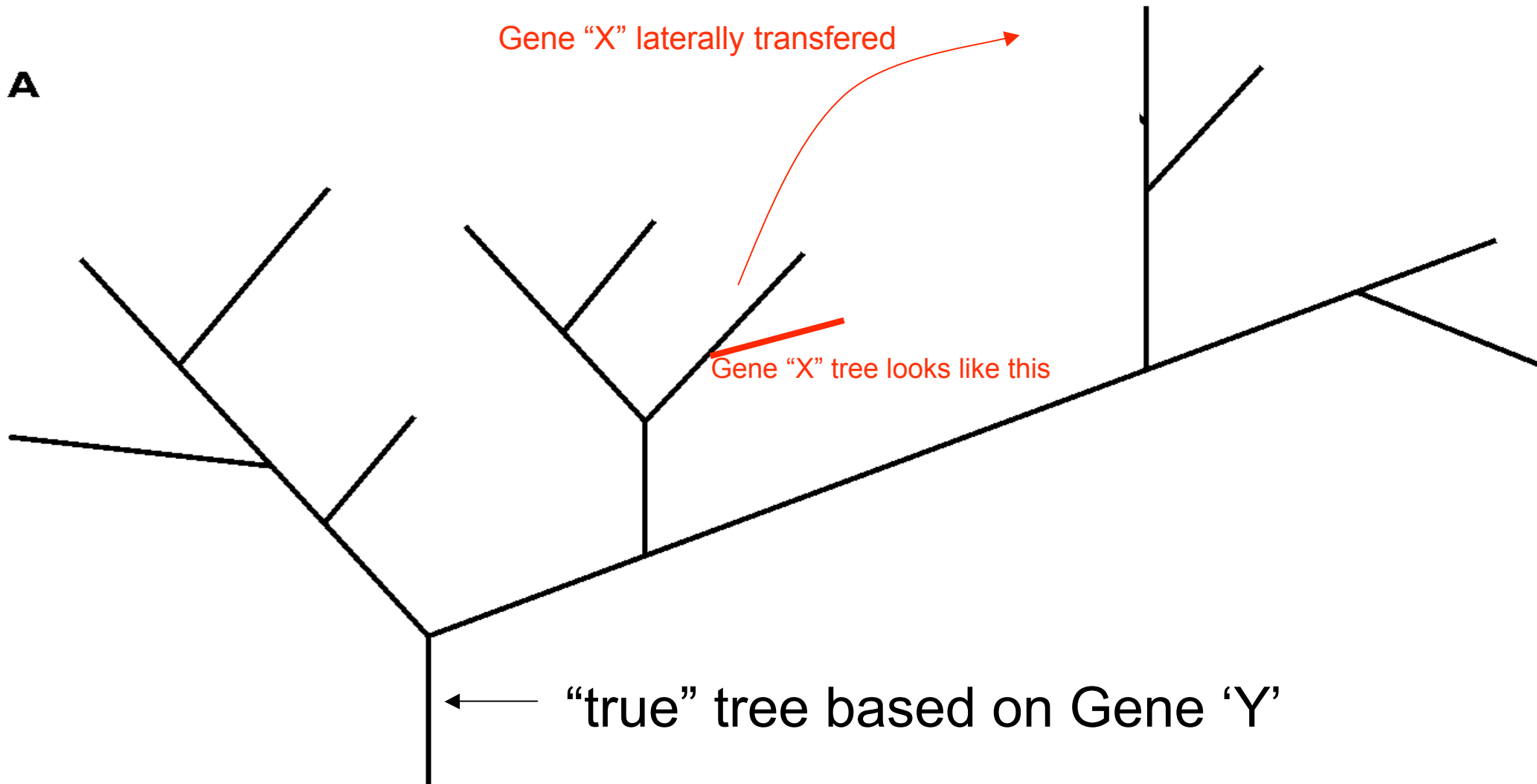
3. Construct tree from pairwise distances

Lateral Gene Transfer and molecular phylogenetics

A

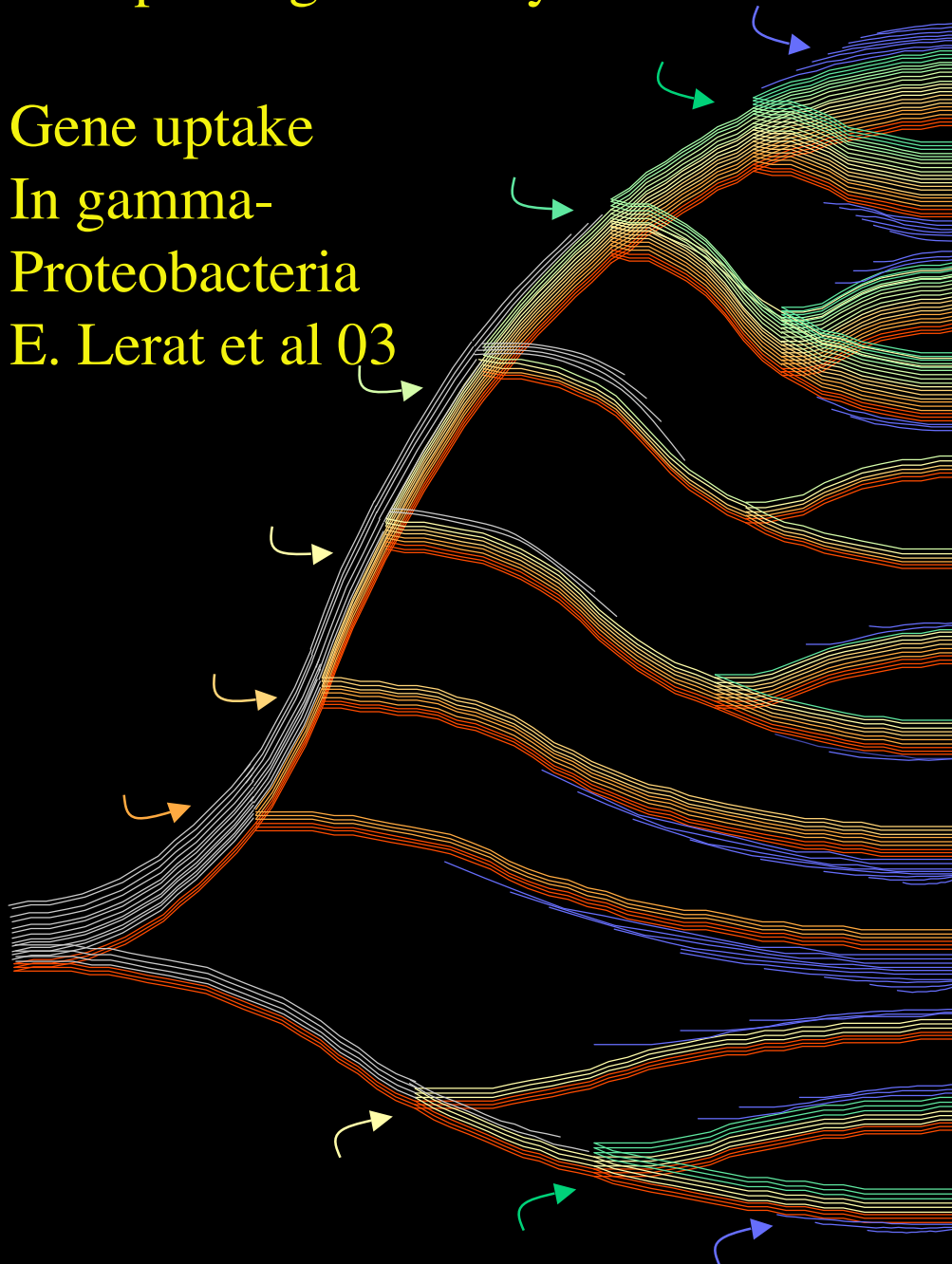
"Gene tree" assumes similarity by descent

But what if there is extensive lateral gene transfer between bacteria ???

A

Gene "X" laterally transfered

Gene "X" tree looks like this

"true" tree based on Gene 'Y'

Example of genome dynamics over time due to Lateral Gene Transfer

Gene uptake
In gamma-
Proteobacteria
E. Lerat et al 03

S. typhimurium

E. coli

Y. pestis KIM

Y. pestis CO92

W. brevipalpis

B. aphidicola

P. multocida

H. influenzae

V. cholerae

P. aeruginosa

X. fastidiosa

X. campestris

X. axonopodis

Many genes in most genomes arrived via LGT after the common ancestor.

Most genes arriving via LGT come from distant sources (not in this group)

*Many persist as vertically transmitted genes within the descendant clade.
---but many are lost quickly (many present only in tips of tree)*

Detecting Horizontal Transfers

1. Unexpected ranking of sequence similarity among homologs
2. Unexpected phylogenetic tree topology
3. Unusual phyletic pattern
4. Conservation of gene order
5. Anomalous DNA composition

"All criteria for identifying probable horizontal gene transfer, or more precisely acquisition of foreign genes by a particular genome, inevitably rely on some unusual feature(s) of subsets of genes that distinguishes them from the bulk of genes in the genome." Koonin et al. 2001

- Direct proofs are unavailable
- Indications of horizontal transfers remain probabilistic

Image of an aphid (Acyrthosiphon pisum) removed due to copyright restrictions.

Essential amino acids - not in the bugs diet !

Name	Recommended daily intake in human Adults mg per Kg body weight WHO	for 70Kg human (mg)
F Phenylalanine	14 (sum with Tyrosine)	980
L Leucine *	14	980
M Methionine	13 (sum with Cysteine)	910
K Lysine	12	840
I Isoleucine	10	700
V Valine	10	700
T Threonine *	7	490
W Tryptophan	3	245
H Histidine	unknown, 28 in infants (? sum with arginine)	(? 1960)
R Arginine	unknown, required for infants, maybe seniors	(?)

Plasmids for essential amino acid biosynthesis found in aphid symbionts

Figure by MIT OCW.
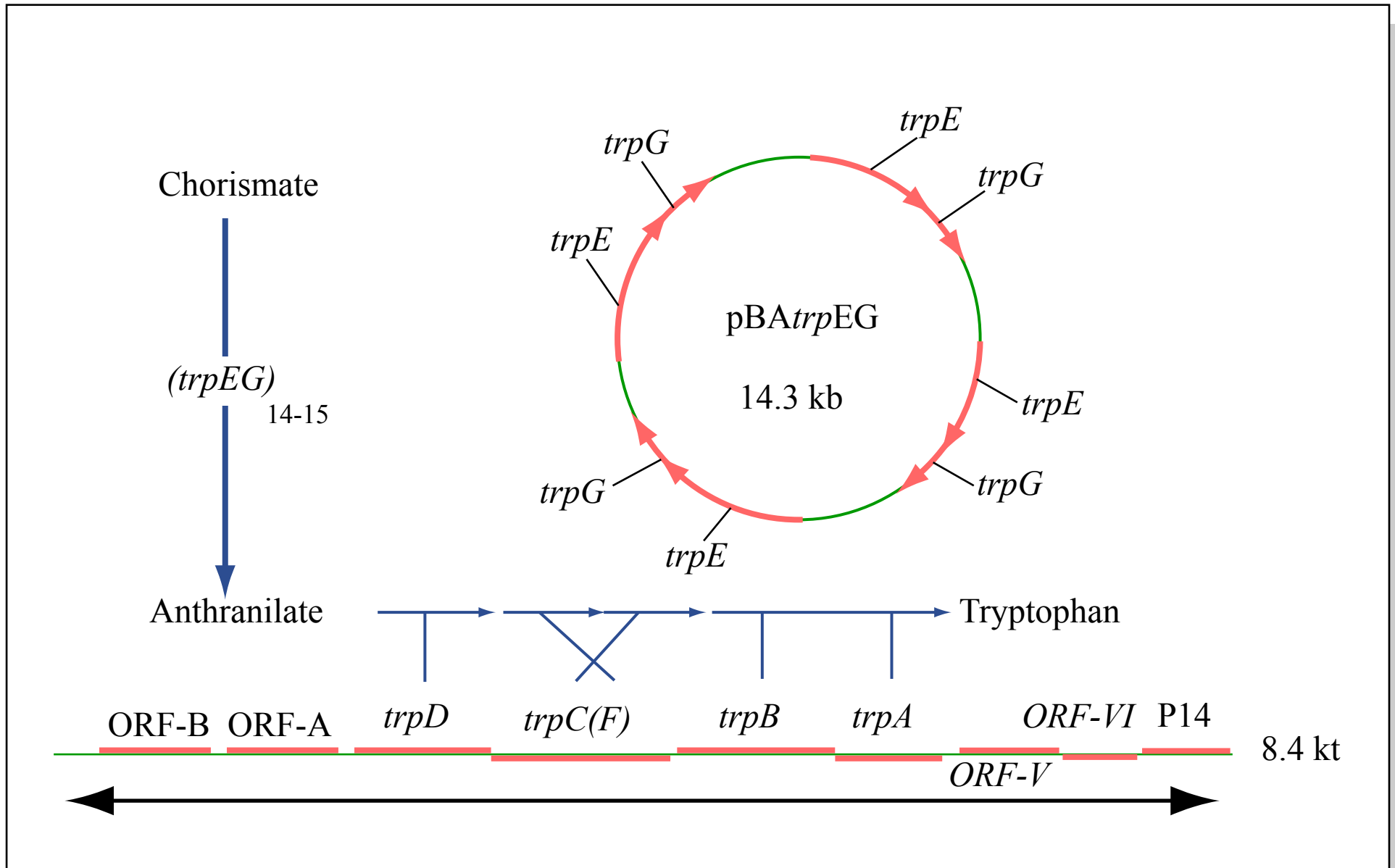
Aphid Host	Aphid Clone/Population	*leuABCD*	*trpEG*
A. pisum	12 United Kingdom clones	—	2.4-16.2*
	N. A. Moran lab clone 5A (Madison, Wl)	0.6	4.8
Diuraphis noxia	P. Baumann lab clone (Lincoin, NE)	0.9	1.8
	South Africa population	0.3	0.4
Rhopalosiphum maidis	N. A. Moran lab clone (Tucson)	—	0.3
S. graminum	Biotype B (K. A. Shufran lab clone)	—	0.5
	Biotype E (T. Mittler lab clone)	23.5	14.5
	Biotype E (P. Baumann lab clone)	1.4	2.1
	Biotype E (N. A. Moran lab clone)	1.9	1.5
	Biotype E (K.A. Shufran lab clone)	1.6	2.6
	Biotype G (K.A. Shufran lab clone)	0.5	2.4
	Biotype SC (K.A. Shufran lab clone)	—	0.5
Uroleucon ambrosiae	86 individuals, 15 U.S. populations	0.5-2.8	0.3-1.9

The ratios of copies of plasmid-borne amino acid biosynthetic genes (leuABCD, trpEG) to chromosomal gene copies for Buchnera of different aphid species and strains.

Figure by MIT OCW.

Moran et al., PNAS 100:14545 (2003)

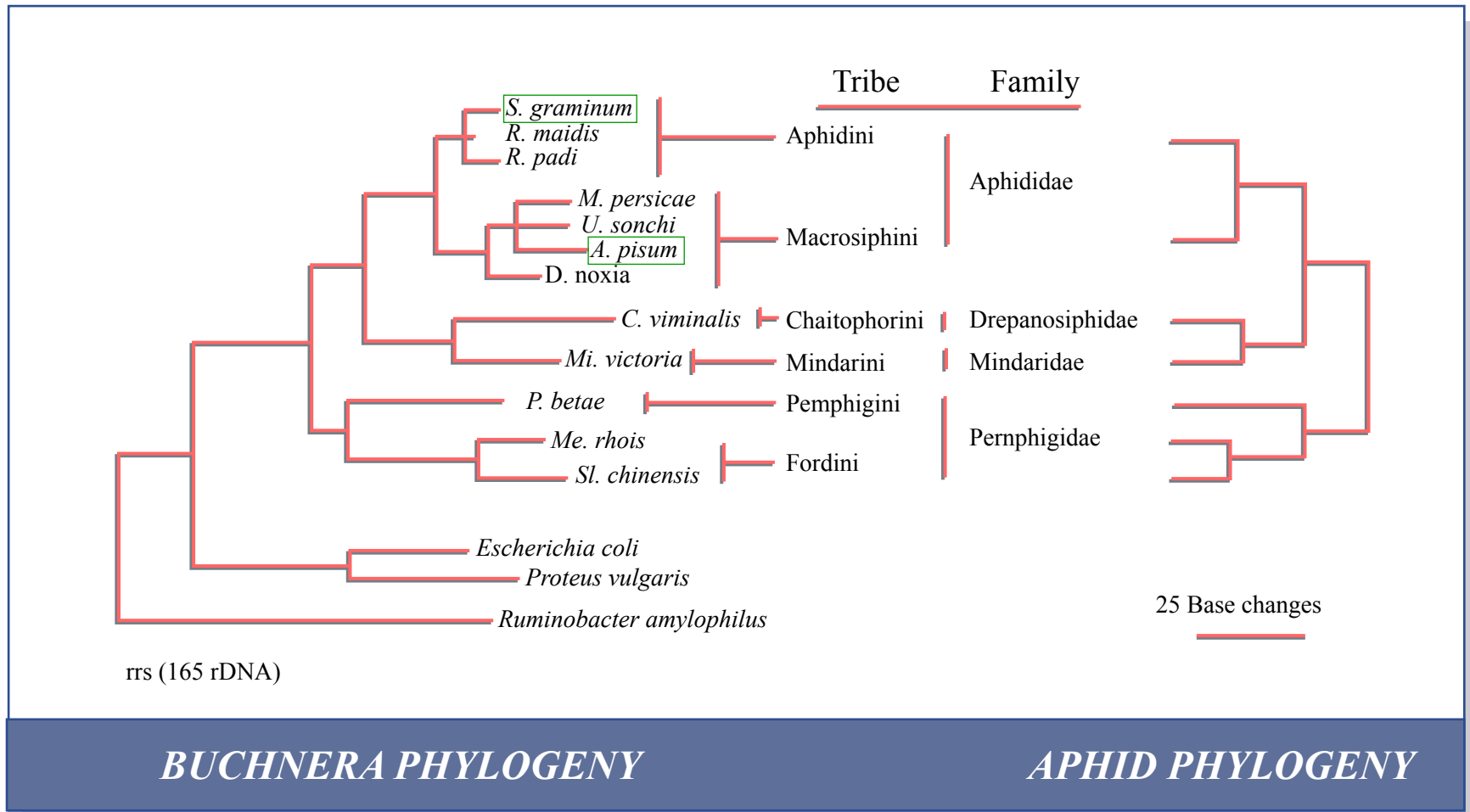# Symbiont phylogeny mirrors insect host phylogeny - co-evolution

Tribe   Family

S. graminum
R. maidis
R. padi   Aphidini

                                       Aphididae

M. persicae
U. sonchi
A. pisum   Macrosiphini
D. noxia

C. viminalis   Chaitophorini   Drepanosiphidae
Mi. victoria   Mindarini   Mindaridae

P. betae   Pemphigini
Me. rhois   Pernphigidae
Sl. chinensis   Fordini

Escherichia coli
Proteus vulgaris

Ruminobacter amylophilus

rrs (165 rDNA)

25 Base changes

BUCHNERA PHYLOGENY   **APHID PHYLOGENY**

Figure by MIT OCW.

Figures removed due to copyright restrictions.

UNCHARACTERIZED NATIVE TAXA ⟶ GENOMIC ANALYSES ?

Figures removed due to copyright restrictions.

Haemophilus influenzae
Fleischmann,R.D et al. 1995 Science 269: 496-512

Two basic metagenomic approaches

1. Extract DNA from environmental sample

2. Construct library

conventional small insert (<10kb) library

large insert (cosmid or BAC) library (up to 200 kb), allows sampling of whole operons

3. Screen

Sequence DNA or RNA, look for genes with functions of interest

Perform functional screens: directly test for some biochemical property in the cloning host

Limitations:

Limits search to genes with detectable (evolutionary) homology to functionally characterized genes: Sequence or structural homology

Possible problems with efficient transcription of the cloned fragment, translation, secretion of the product, correct chaperones for folding of the product
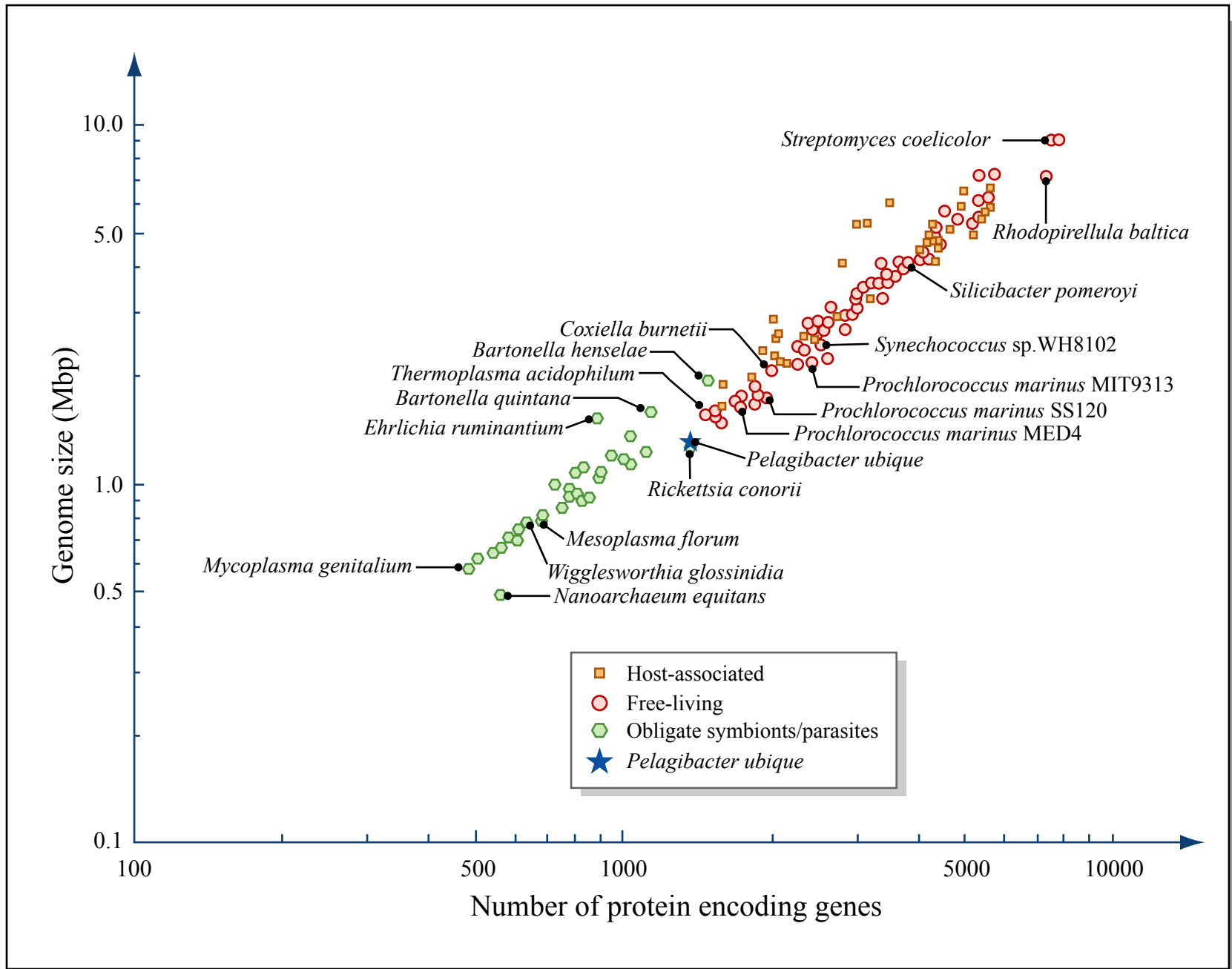
Figure by MIT OCW.

Genome sequence of the endocellular bacterial symbiont of aphids Buchnera sp. APS Shuji Shigenobu, Hidemi Watanabe, Masahira Hattori, Yoshiyuki Sakaki, Hajime Ishikawa

Nature **407**, 81-86 (07 Sep 2000)

Figure removed due to copyright restrictions.

The 160-Kilobase Genome of the Bacterial Endosymbiont *Carsonella*

Atsushi Nakabachi,[1,2*] Atsushi Yamashita,[2†] Hidehiro Toh,[3,4†] Hajime Ishikawa,[5] Helen E. Dunbar,[2] Nancy A. Moran,[2] Masahira Hattori[6,7*]

Figure removed due to copyright restrictions.

2000 genes lost from ancestor, to Sg/Ap divergence
Must have been rapid evolution and gene loss !

Diagram showing Buchnera gene loss from a reconstructed
enteric ancestor removed due to copyright restrictions.

COMPARATIVE GENOMICS

50 Million Years of Genomic Stasis in Endosymbiotic Bacteria

Ivica Tamas,[1]* Lisa Klasson,[1]* Björn Canbäck,[1]
A. Kristina Näslund,[1] Ann-Sofie Eriksson,[1]
Jennifer J. Wernegreen,[2] Jonas P. Sandström,[1] Nancy A. Moran,[2]
Siv G. E. Andersson[1]†

Comparison of Genome Features for *B. aphidicola* (Sg) and *B. aphidicola* (Ap)

Feature	*B. aphidicola* (Sg)	*B. aphidicola* (Ap)
Genome size (bp)	641, 454	640, 681
Genic G + C content (%)	26.2	26.3
Intergenic G + C content (%)	14.8	16.1
Protein coding genes (no.)	545	564
Pseudogenes (no.)	38	13
Avg. gene length (bp)	978	985
Avg. intergenic length (bp)	118	127

Figure by MIT OCW.

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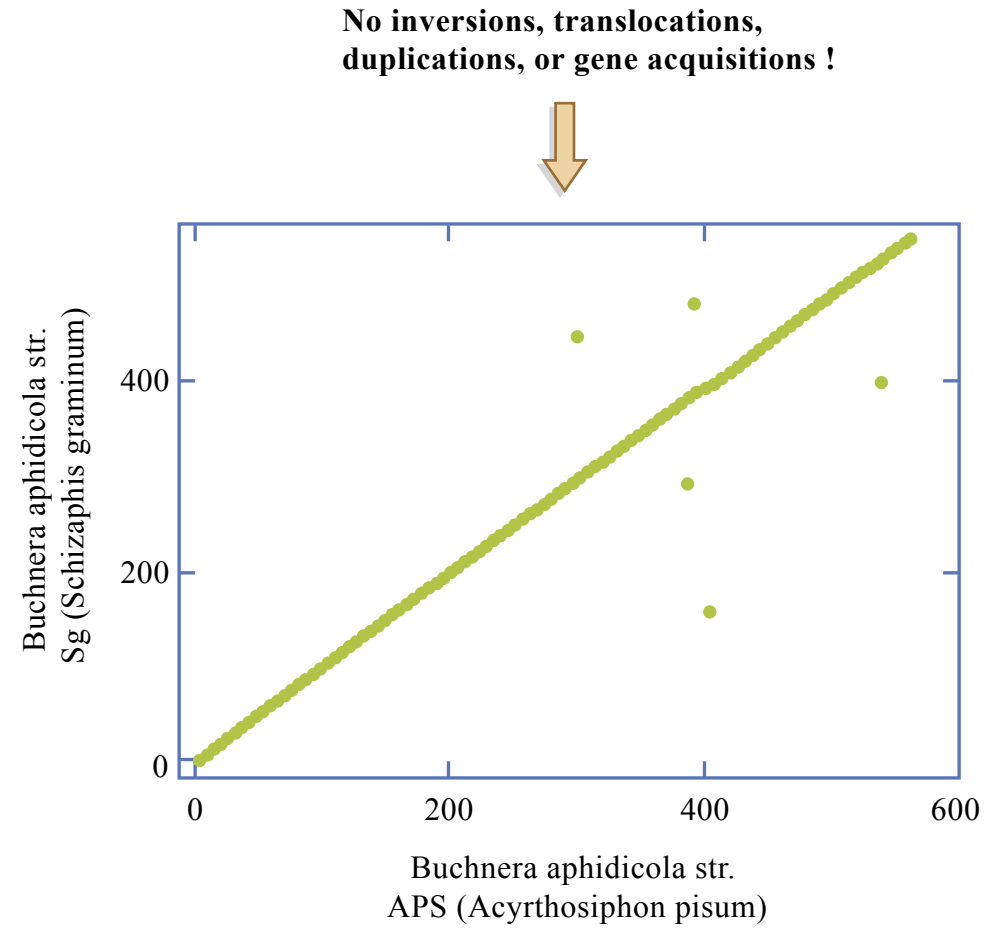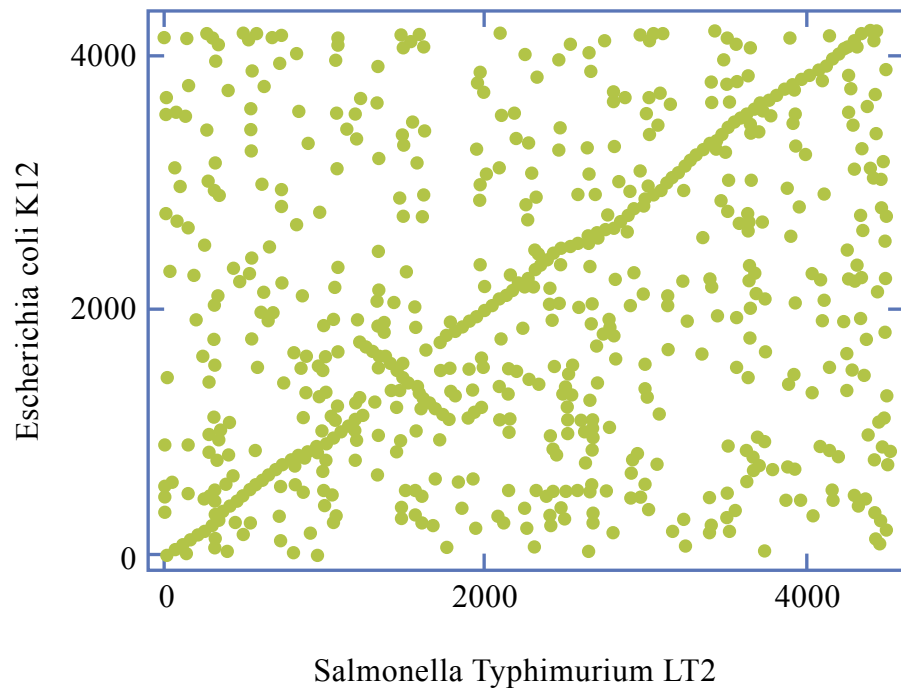Siv G. E. Andersson[1]†

Buchnera aphidicola the bacterial symbiont, was compared (strain-wise) between
the aphids *Schizaphus graminum* (Sg) versus *Acyrthosiphon pisum* (Ap)

After 70 million years, **no chromosomal rearrangements or gene acquisitions**

But considerable sequence divergence, and substantial gene loss
(9e-9 **synonymous** substitutions/yr; 1.65e-9 **non-synonymous** substitutions/yr)

In comparison, *E coli* vs. *S. typhyimurium:* 2000X more labile in gene content/orde

Gene plot, ncbi
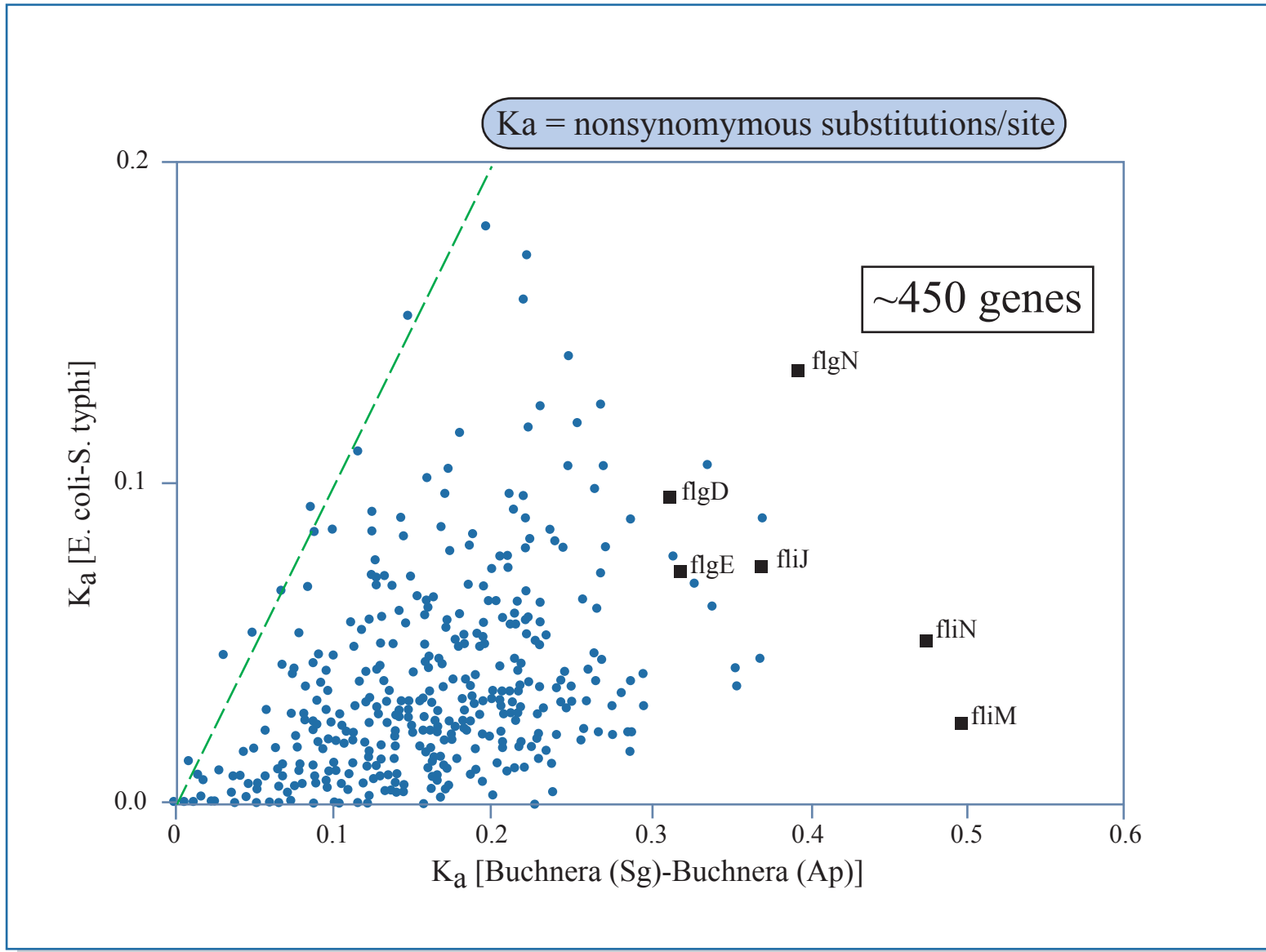

Figure by MIT OCW.

Ka = nonsynomymous substitutions/site

~450 genes

K_a [E. coli-S. typhi]

K_a [Buchnera (Sg)-Buchnera (Ap)]

flgN
flgD
flgE
fliJ
fliN
fliM

Figure by MIT OCW.

y-Proteobacteria

0.065

100 — Buchnera (Sg)
Buchnera (Ap)
100
100 — S. typhi
100 — S. typhimurium
E. coli K12
99 — E. coli 0157

100 — R. prowazekii
R. conorii

100 — H. pylori 26695
H. pylori J99

100 — B. subtilis
B. halodurans

100 — M. pneumoniae
M. genitalium

100 — C. pneumoniae AR39
100 — C. muridarum
C. trachomatis

Free-living

Obligate Symbiont

K12-O157
St-Sty
7
8
9
6
5
3 4
1
Bu 2

log(Rearrangement/Ka)
5
4
3
2
1
0

0 25 50 75 100 125 150
Repeats (kb)

Genome size (Mb)
1 2 3 4 5 6

K12-O157
St-Sty
7
8
9
6
5
3,4
1
2
Bu

log(Indel/Ka)
6
5
4
3
2
1
0

0 25 50 75 100 125 150
Repeats (kb)

Genome size (Mb)
1 2 3 4 5 6

Figure by MIT OCW.

The 160-Kilobase Genome of the Bacterial Endosymbiont *Carsonella*

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Science 314:267
(Oct 13, 2006)
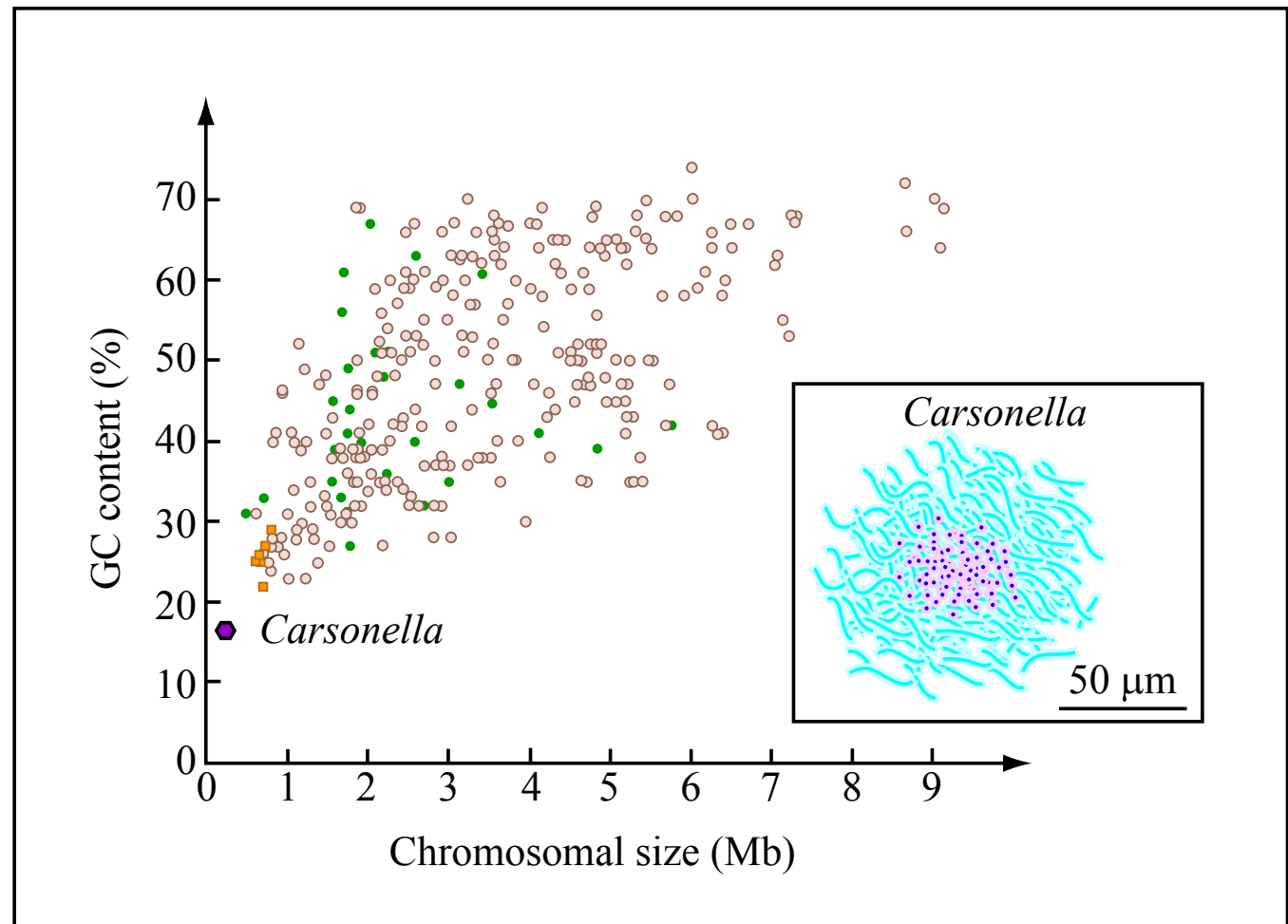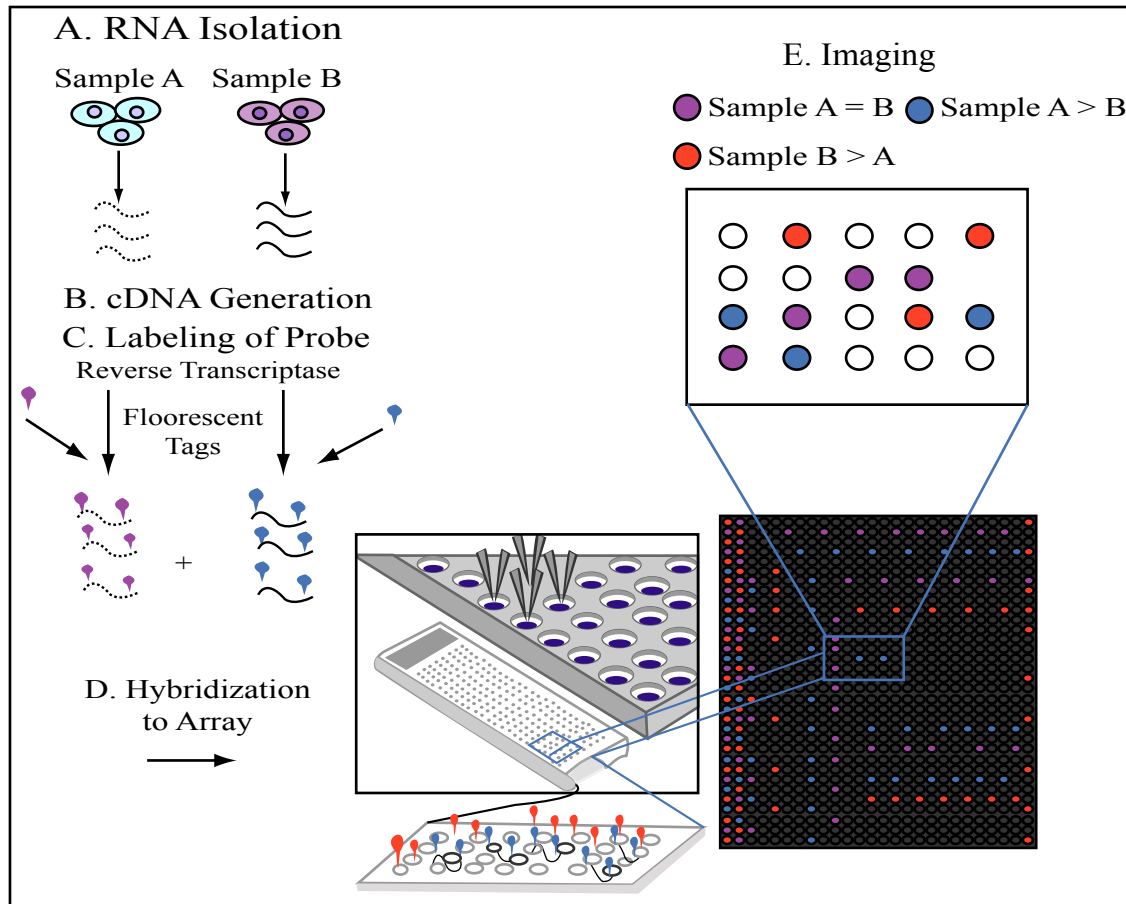
GENOME DYNAMICS IN *Buchnera*

- Enhanced stability of genome architecture in obligate symbionts, despite substantial sequence divergence

- Prominence of pseudogenes, loss of DNA repair mechanisms (So how is genome stability maintained ???)

- Gene transfer elements are greatly reduced/eliminated (Reduced phage, exchange with other genomes, repeat seqs, transposons)

- Lack of recombination mechanisms (no recA and recF) - lowers rearrangement/gene acquisitions

- Lowered freqs of recombin., likely renders it neutrally selective => genome stasis

A. RNA Isolation

Sample A   Sample B

B. cDNA Generation
C. Labeling of Probe
Reverse Transcriptase

Floorescent Tags

+

D. Hybridization to Array

E. Imaging

● Sample A = B   ● Sample A > B
● Sample B > A

- Prepare (or buy) microarray carrying "probes" of interest

- Isolate RNA from cells

- Generate cDNA and label "targets"

- Samples will be labeled with two different fluorescent dyes

- Incubate this hybridization mix with DNA microarrays

- Scan microarrays to detect bound cDNA. Store data

- Analyze data

Figure by MIT OCW.

Graphs removed due to copyright restrictions.

A genomic perspective on nutrient provisioning by bacterial symbionts of insects

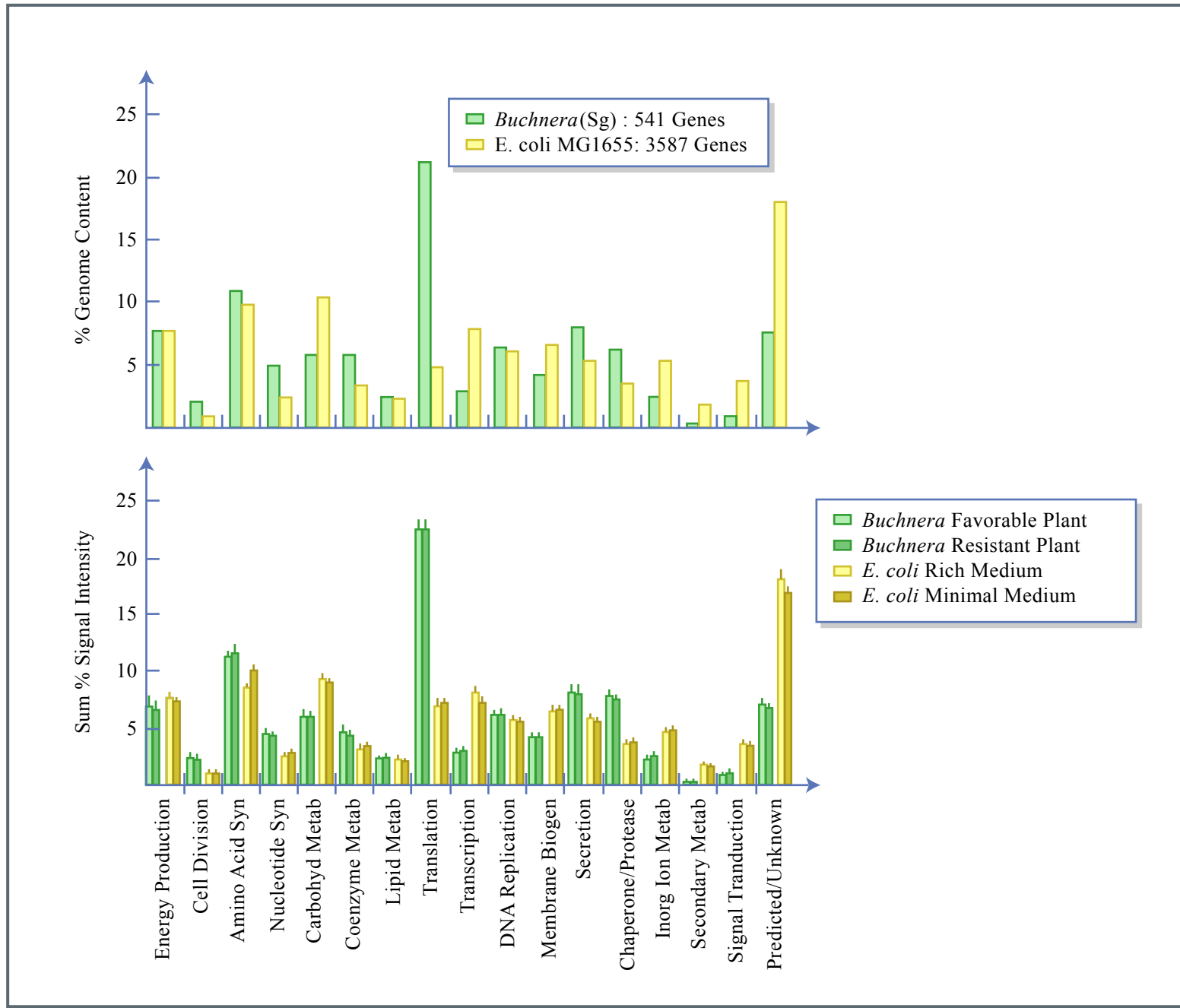Nancy A. Moran*[†‡], Gordon R. Plague*[†], Jonas P. Sandström[§], and Jennifer L. Wilcox*

Figure by MIT OCW.

A genomic perspective on nutrient provisioning by bacterial symbionts of insects   PNAS 100:14543 (2003)

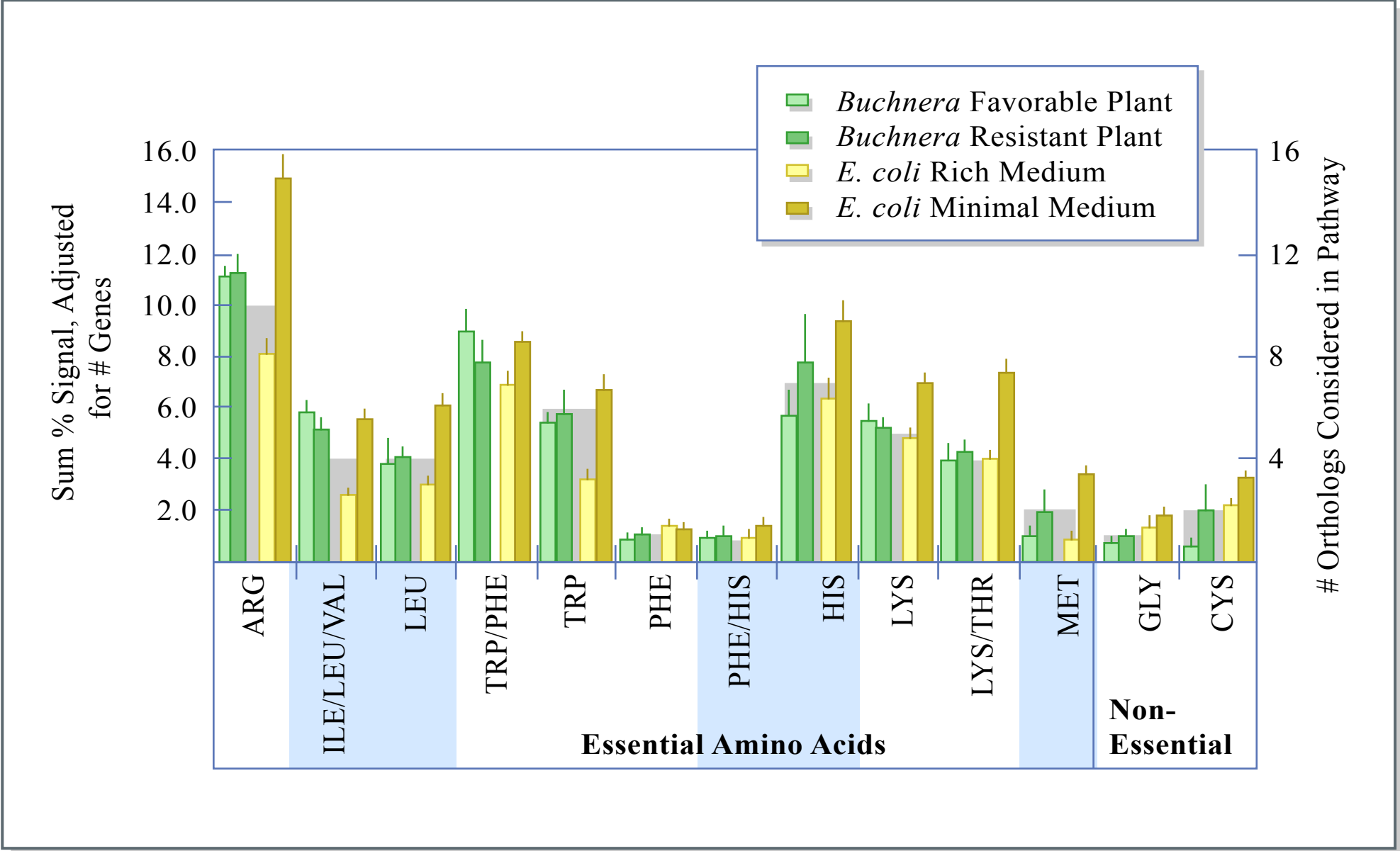Nancy A. Moran*[†‡], Gordon R. Plague*[†], Jonas P. Sandström[§], and Jennifer L. Wilcox*

Figure by MIT OCW.

Two different bacterial endsymbionts in glassy-winged sharpshooter bacteriome

Microscopic image of Baumannia and Sulcia removed due to copyright restrictions.

Metabolic Complementarity and Genomics of the Dual Bacterial Symbiosis of Sharpshooters

Dongying Wu, et al. PLoS Biology 4:(6) e188 (2006)

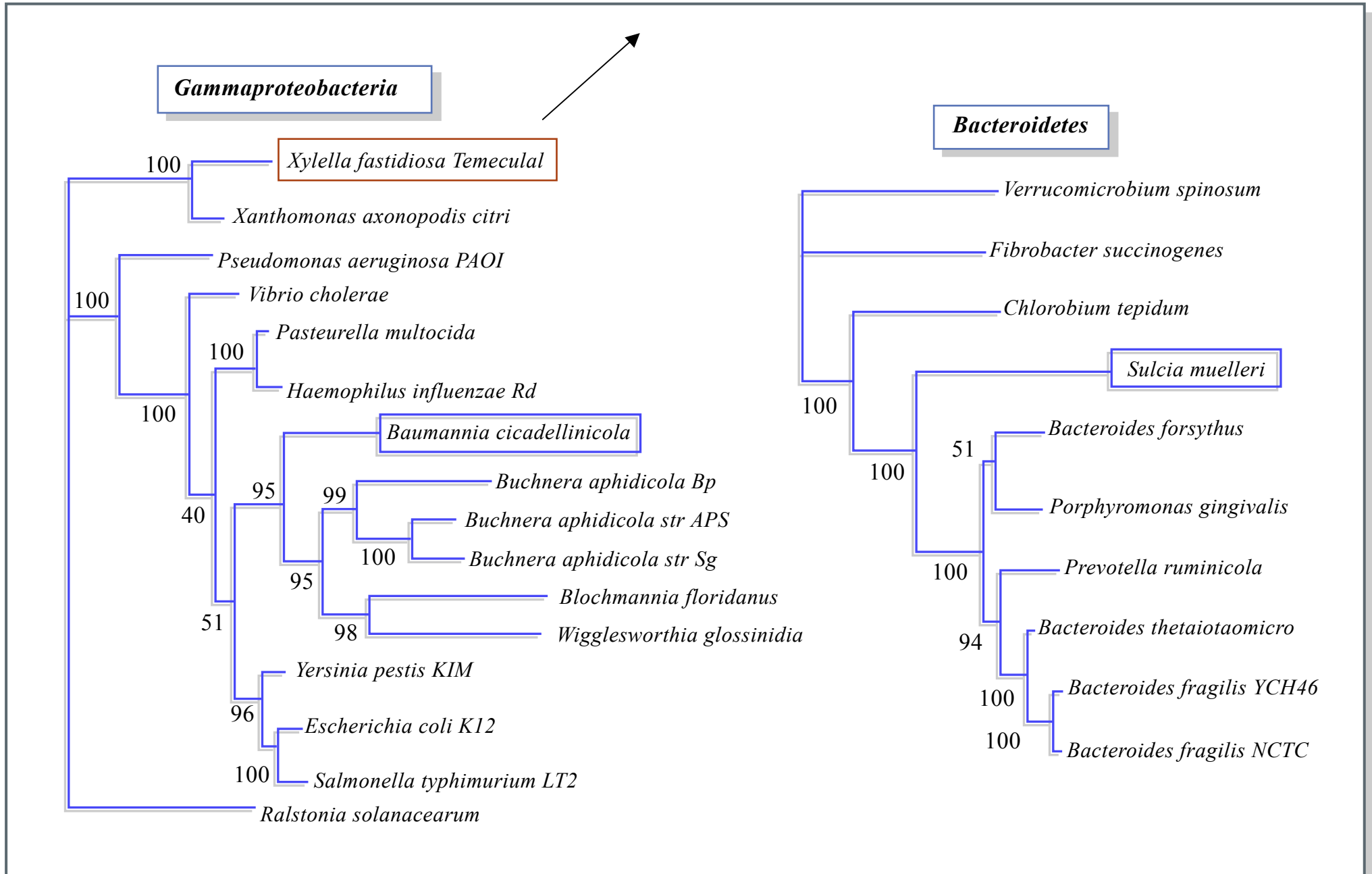Sharpshooters are also a vector for extracellular *Xyllela* a pathogen on grapes

Gammaproteobacteria

- *Xylella fastidiosa Temecula1* — 100
- *Xanthomonas axonopodis citri*
- *Pseudomonas aeruginosa PAOI* — 100
- *Vibrio cholerae* — 100
- *Pasteurella multocida* — 100
- *Haemophilus influenzae Rd*
- *Baumannia cicadellinicola* — 95
- *Buchnera aphidicola Bp* — 99
- *Buchnera aphidicola str APS* — 100
- *Buchnera aphidicola str Sg* — 95
- *Blochmannia floridanus* — 98
- *Wigglesworthia glossinidia*
- *Yersinia pestis KIM* — 96
- *Escherichia coli K12* — 100
- *Salmonella typhimurium LT2*
- *Ralstonia solanacearum*
- 40, 51

Bacteroidetes

- *Verrucomicrobium spinosum*
- *Fibrobacter succinogenes*
- *Chlorobium tepidum* — 100
- *Sulcia muelleri*
- *Bacteroides forsythus* — 51, 100
- *Porphyromonas gingivalis*
- *Prevotella ruminicola* — 100
- *Bacteroides thetaiotaomicro* — 94
- *Bacteroides fragilis YCH46* — 100
- *Bacteroides fragilis NCTC* — 100