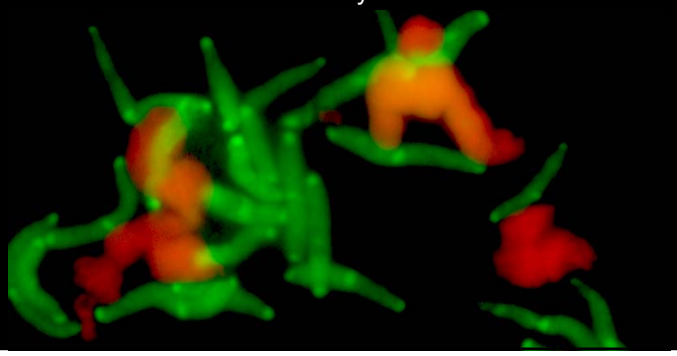
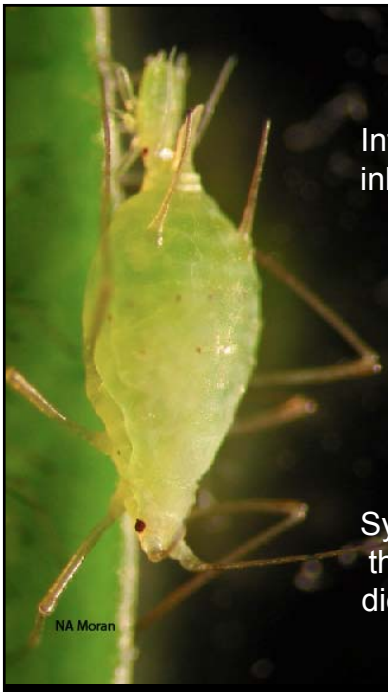
Genomics and Functions of the Two Bacterial Symbionts of Sharpshooters

Nancy A. Moran and John McCutcheon

Department of Ecology and Evolutionary Biology
& Center for Insect Science
The University of Arizona





Nutritional symbiosis in insects:

Intimate association with bacteria that inhabit cells of a specialized organ, or "bacteriome"

Obligate for host and symbiont

Inherited from mother to progeny

Evolutionarily ancient

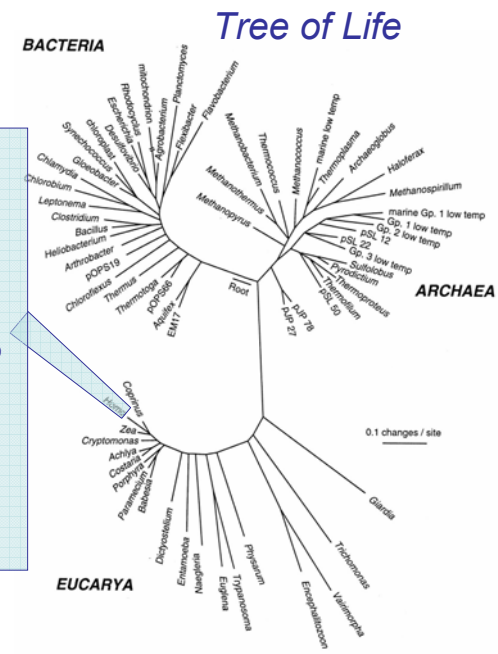
Symbiont provisions needed nutrients that are rare or absent in specialized diets such as phloem sap, xylem sap, vertebrate blood

Animal Genomes:

Ancestral loss of many genes:

-genes underlying biosynthesis of essential metabolites (vitamins, amino acids)

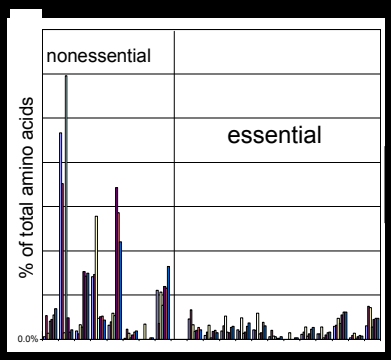
CONSEQUENCE: Animals, including mammals and insects, have complex nutritional needs



N Pace

Essential amino acids are rare in diet of insects feeding on phloem sap or xylem sap

Amino acid abundances in phloem sap of 6 species of angiosperms

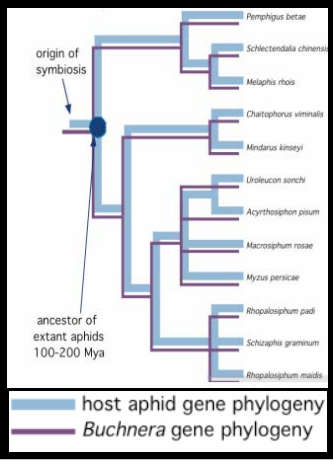


Buchnera aphidicola,
"primary" symbiont of aphids
Model of nutritional symbiosis

Ancient infection,
strictly vertical transmission

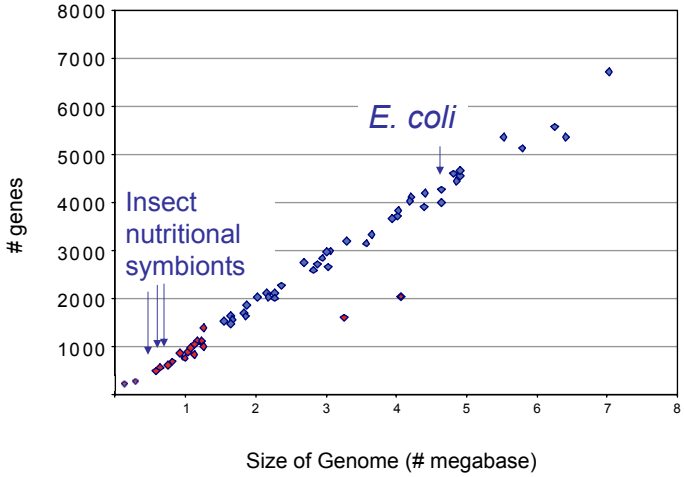
Very small genome, 600 kilobases

Shigenobu et al 2000 Nature



In Bacteria: a small genome implies few genes

- *=obligate symbiont or pathogen*



The *Buchnera* gene set reflects its nutritional role.

Essential amino acid biosynthetic pathways

argA argB argC argD argE *carAB argF argG argH*
Glutamate---> ---> ---> ---> ---> Ornithine ---> ---> ---> ---> ARG

ilvHI ilvC ilvD ilvE
Pyruvate ---> ---> ---> ---> VAL

ilvA *ilvHI ilvC ilvD ilvE*
Threonine ---> a-Ketobutyrate ---> ---> ---> ---> ILE
+ Pyruvate

ilvHI ilvC ilvD leuA leuCD leuB ilvE
Pyruvate ---> ---> ---> ---> ---> ---> ---> LEU

aroH aroB aroD aroE aroK aroA aroC
PEP+Erythrose ---> ---> ---> ---> ---> ---> ---> Chorismate
4-Phosphate

pheA pheA hisC
Chorismate ---> ---> ---> PHE

trpEG trpD trpC trpC trpAB
Chorismate ---> ---> ---> ---> ---> TRP

thrA asd thrA *thrB thrC*
Aspartate ---> ---> ---> Homoserine ---> ---> THR
metB metC metE
Homoserine ---> ---> ---> MET

thrA asd dapA dapB dapD dapC dapE dapF lysA
Aspartate ---> ---> ---> ---> ---> ---> ---> ---> ---> LYS

hisG hisI hisA hisHF hisB hisC hisB hisD
PRPP + ATP ---> ---> ---> ---> ---> ---> ---> ---> HIS

Nonessential amino acid biosynthetic pathways

tyrA tyrA hisC
Chorisimate ---> ---> ---> TYR

proB proA proC
Glutamate ---> ---> ---> PRO

serA serC serB
3-Phosphoglycerate ---> ---> ---> SER

glyA
Serine ---> GLY

cysE cysK
Serine ---> ---> CYS

gtBD/gdhA
2-oxoglutarate ---> GLU

glnA
Glutamate ---> GLN

aspC+tyrB
Oxaloacetate ---> ASP

asnB/asnA
Aspartate ---> ASN

alaB/avtA
Pyruvate ---> ALA

GENE present in *Buchnera* (Ap)

GENE absent in *Buchnera* (Ap)

(based on Shigenobu et al 2000)

Sharpshooters

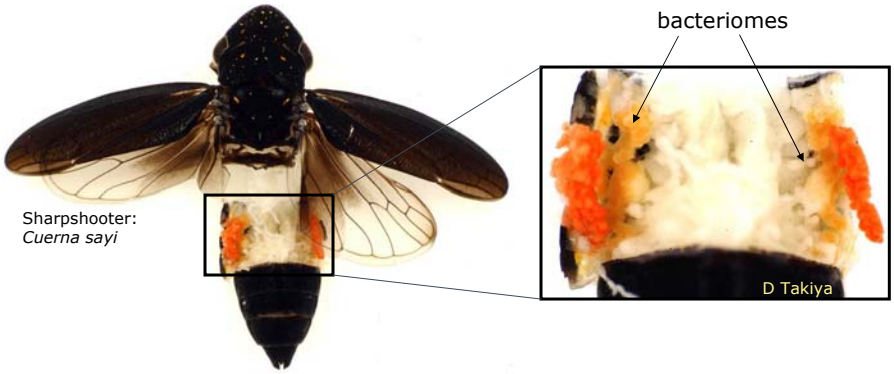
Family Cicadellidae:

- 25,000 spp.: largest family of sap-sucking herbivores
- Most subfamilies feed on phloem sap, rich in sugars and other organic compounds, unbalanced amino acid composition

Subfamily Cicadellinae:

- 2,500 spp.
- xylem sap specialist
- Nutrition especially poor

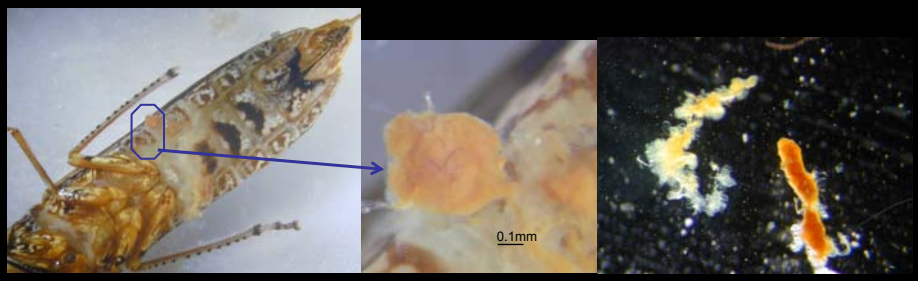
Sharpshooters harbor two obligate symbionts in their bacteriomes



Candidatus "*Baumannia cicadellinicola*" (Gammaproteobacteria)

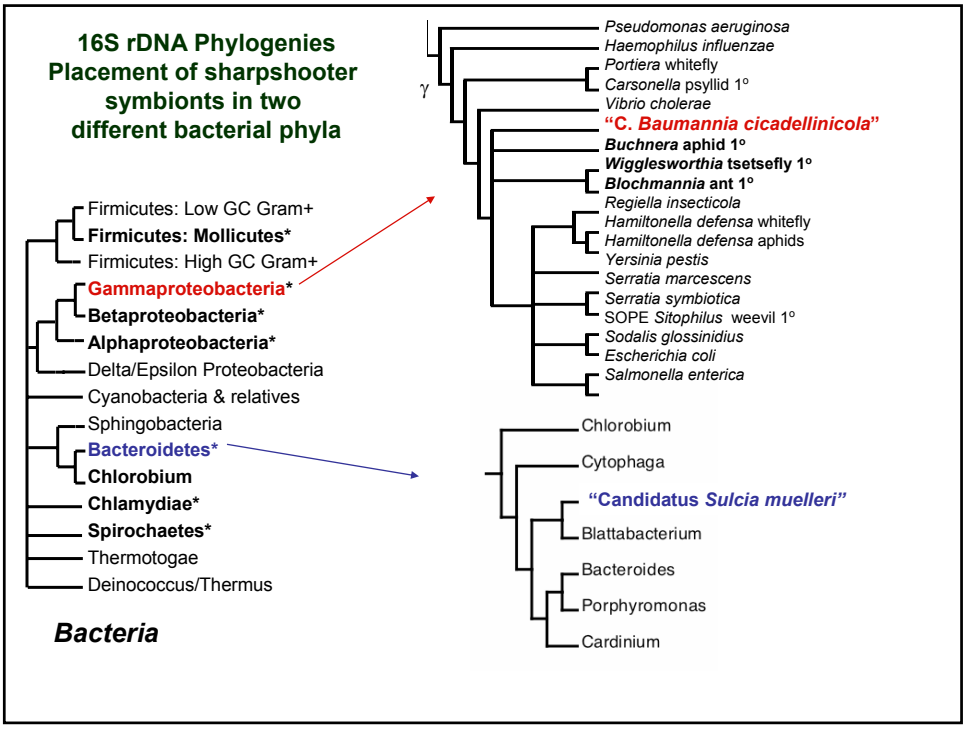
Candidatus "*Sulcia muelleri*" (Bacteroidetes)

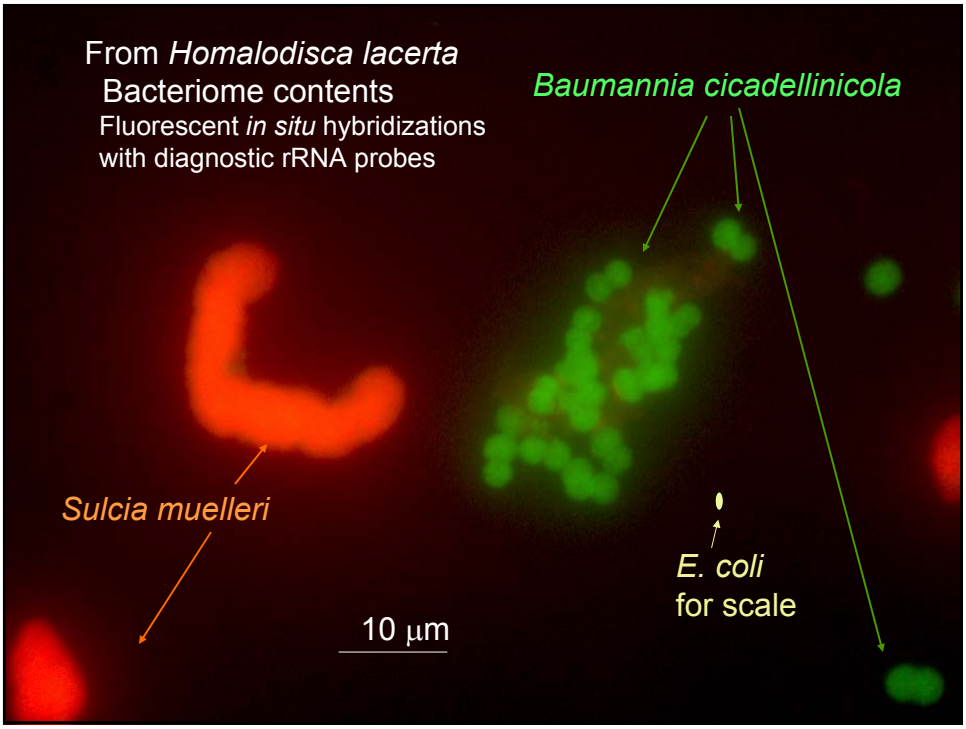
Moran *et al*. 2003 *Environ. Microbiol.*
Moran *et al.* 2005 *Appl. Environ. Microbiol.*



Bacteriome dissected from anterior abdomen of *H. vitripennis*
Orange-red portion- *Baumannia* only
Yellow portion- *Baumannia* and *Sulcia*

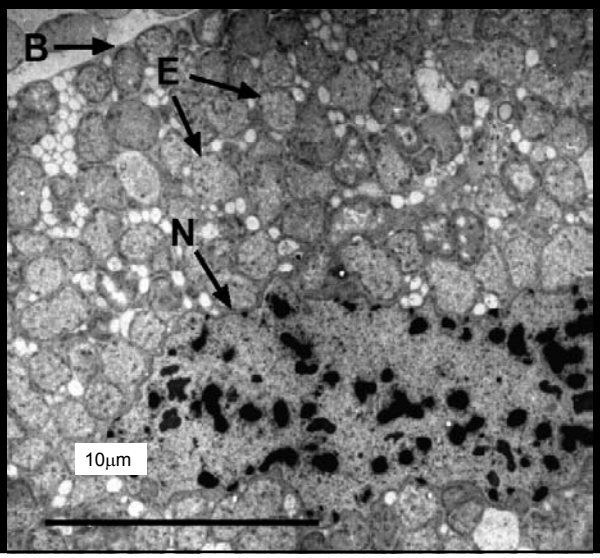
(Moran et al. 2003 *Environmental Microbiology*)


16S rDNA Phylogenies
Placement of sharpshooter symbionts in two different bacterial phyla
Firmicutes: Low GC Gram+
Firmicutes: Mollicutes*
Firmicutes: High GC Gram+
Gammaproteobacteria*
Betaproteobacteria*
Alphaproteobacteria*
Delta/Epsilon Proteobacteria
Cyanobacteria & relatives
Sphingobacteria
Bacteroidetes*
Chlorobium
Chlamydiae*
Spirochaetes*
Thermotogae
Deinococcus/Thermus
Bacteria
γ
Pseudomonas aeruginosa
Haemophilus influenzae
Portiera whitefly
Carsonella psyllid 1°
Vibrio cholerae
"C. Baumannia cicadellinicola"
Buchnera aphid 1°
Wigglesworthia tsetsefly 1°
Blochmannia ant 1°
Regiella insecticola
Hamiltonella defensa whitefly
Hamiltonella defensa aphids
Yersinia pestis
Serratia marcescens
Serratia symbiotica
SOPE Sitophilus weevil 1°
Sodalis glossinidius
Escherichia coli
Salmonella enterica
Chlorobium
Cytophaga
"Candidatus Sulcia muelleri"
Blattabacterium
Bacteroides
Porphyromonas
Cardinium


From Homalodisca lacerta
Bacteriome contents
Fluorescent in situ hybridizations
with diagnostic rRNA probes
Baumannia cicadellinicola
Sulcia muelleri
E. coli
for scale
10 μm

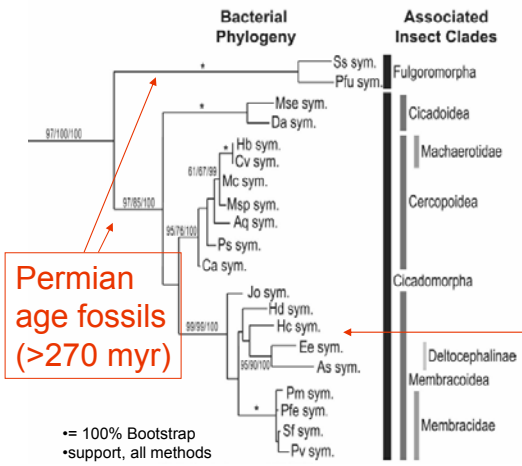
"Candidatus Baumannia cicadellinicola" (*Gammaproteobacteria*)
in "red" portion of bacteriome of *Homalodisca vitripennis*

N=host nucleus B=Bacteriocyte membrane E=Endosymbionts



Irregularly spherical
~2 μm diameter

Phylogeny of *Sulcia muelleri* from Auchenorrhyncha (Hemiptera): the oldest insect symbiont



Permian age fossils (>270 myr)

Broad congruence with host relationships

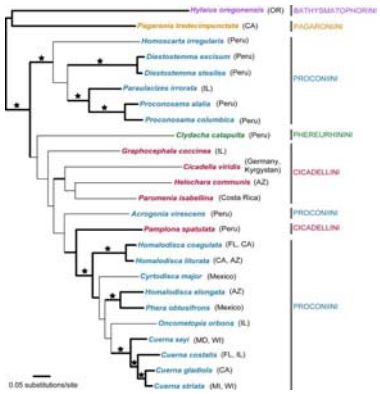
Dates to the origins of vascular plant-feeding in insects

Symbionts derived from sharpshooters

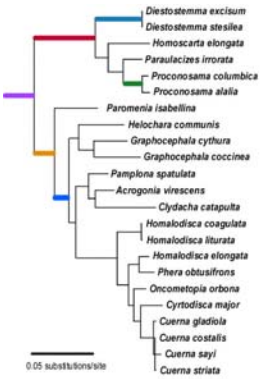
Moran et al. Appl Env Micro 2005

Phylogenetic congruence of sharpshooters and two clades of bacterial symbionts

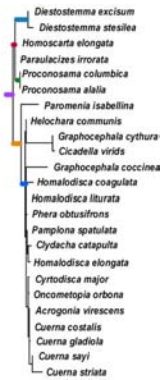
Insect hosts



Baumannia



Sulcia



Statistical tests of codiversification

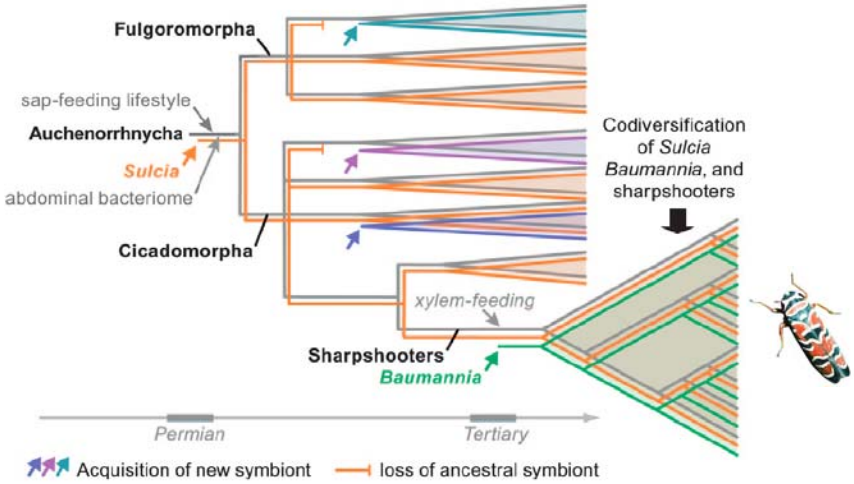
(1) ILD test (2) Shimoidara-Hasegawa test (3) Tree-Fitter

All tests support cospeciation of all three lineages

D. Takiya et al. Mol Ecol. 2006

photos D. Takiya

Dual obligate symbiosis: Sulcia + partner from other phylum



Genome sequencing of sharpshooter symbionts

400 insects from lemon grove in Riverside

Dissect out bacteriomes

↓

Filters to remove most host nuclei

↓

Extract DNA

↓

Small and medium insert libraries

↓

Assemble, sort with blast, GC content

↓

Host, other Contaminants ~48%

↓

Wolbachia <1%

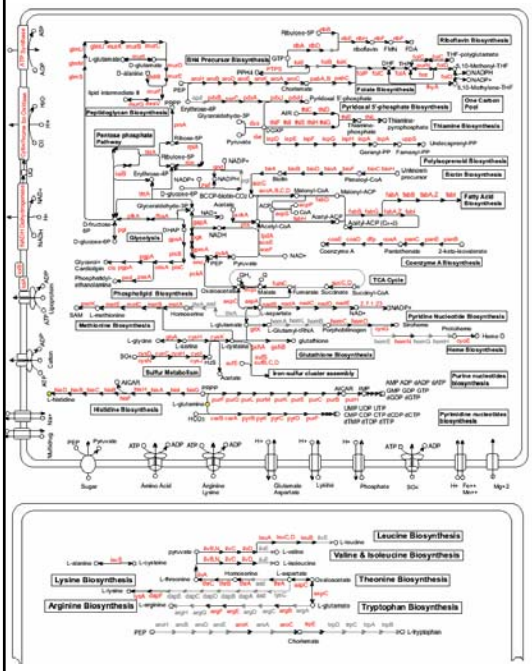
↓

Sulcia ~1% -->Partial genome assembly

↓

Baumannia ~50%-->Full genome assembly

D. Wu, *et al.* 2006 *PLoS Biology*



From genomic sequences of two *H. coagulata* symbionts

Baumannia cicadellinicola
(complete genome sequence)

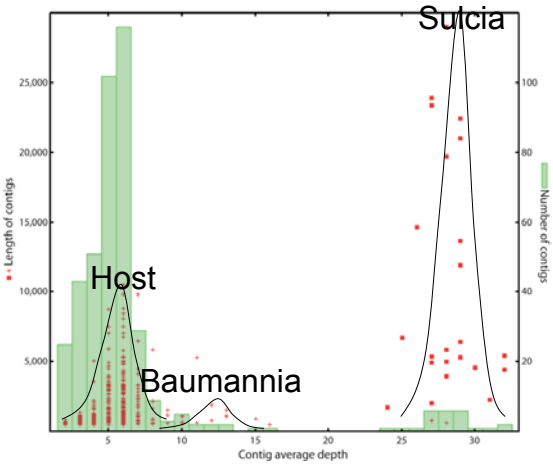
MAKES VITAMINS
Includes pathways for
12 vitamins (84 genes)
One amino acid (His)

Sulcia muelleri
(partial genome sequence, 146 kb)
MAKES AMINO ACIDS
Partial pathways for:
Lys, Trp, Arg, Thr, Val, Ile, Leu

D. Wu et al. PLoS Biology 2006

Completing the *Sulcia* genome
John McCutcheon, postdoc at Arizona

We used 454 GS FLX pyrosequencing on a mixed sample extracted from yellow portion of dissected bacteriomes
26 Mb of sequence assembled into 416 contigs



25 of these contigs were easily separable by depth

23 contigs assembled into one circular genome

Close, but not quite good enough ...

It was great to have a circle, but one problem:
~150 of the homopolymeric regions were wrong because 454 cannot resolve homopolymers.

This was fixed using Illumina/Solexa sequencing technology:
463 Mb (3/8 run) gave ~132X coverage of the *Sulcia* genome

```
                        aaacctaaaaaaaaatattattctttaatattc
                      aaaaaacctaaaaaaaaatattattctttaata
                 tttcaaaaaaaacctaaaaaaaaatattattct
              gtttttcaaaaaaaacctaaaaaaaaatattat
            tgtttttcaaaaaaaacctaaaaaaaaatatta
            tgtttttcaaaaaaaacctaaaaaaaaatatta
          atgtttttcaaaaaaaacctaaaaaaaaatatt
      tttacatgtttttcaaaaaaaacctaaaaaaaa
    tctttaaatgtttttcaaaaaaaacctaaaaaa
   ttctttacatgtttttcaaaaaaaacctaaaaa
 agttctttacatgtttttcaaaaaaaacctaaa
 agttctttacatgtttttcaaaaaaaacctaaa
aagttatttacatgtttttcaaaaaaaacctaa
AAGTTCTTTACATGTTTTTC-AAAAAAACCTAAAAAAAA-TATTATTCTTTAATATTC
```

subset of the Solexa reads

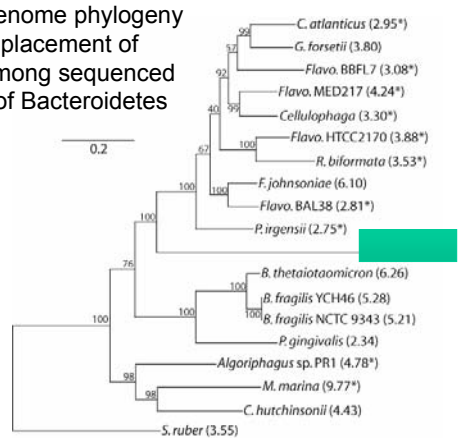
consensus 454 sequence

Sulcia

- smallest genome in Bacteroidetes, 2nd smallest in Bacteria
- first highly reduced symbiont genome outside Gammaproteobacteria

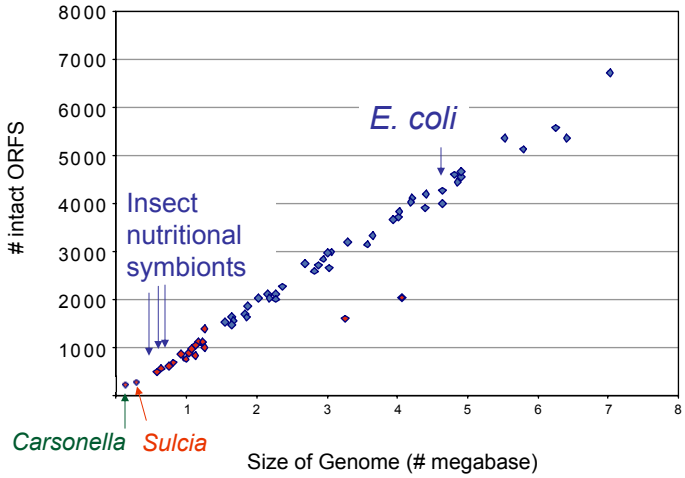
Genome stats:
245,527 bps
22.4% G+C
230 protein genes
31 tRNAs (all aa)
1 rRNA operon
1 tmRNA
96.1% coding density

Whole genome phylogeny showing placement of Sulcia among sequenced species of Bacteroidetes



Genome size and ORF content in some fully sequenced Bacteria

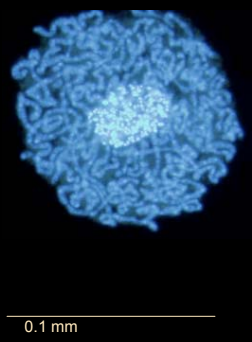
◆ *=intracellular animal associate*



Candidatus Carsonella ruddii (Gammaproteobacteria)
= obligate symbiont of psyllids
degenerate cell morphology, ancient codiversification
Tiniest known genome

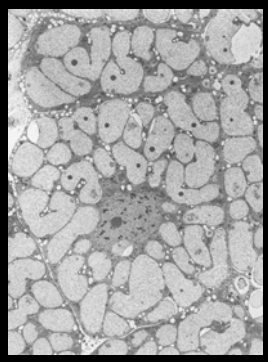
Newly hatched psyllid nymph with bacteriome

N Moran



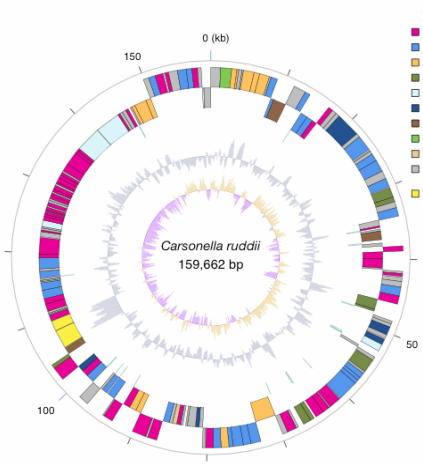
Bacteriocyte showing host nucleus & *C ruddii* cells in cytosol

A. Nakabachi



TEM of bacteriocyte, showing host nucleus & *C ruddii* cells in cytosol

Carsonella ruddii: the *smallest* bacterial genome



COG category
- [J] Translation, ribosomal structure and biogenesis
- [E] Amino acid transport and metabolism
- [C] Energy production and conversion
- [O] Posttranslational modification, protein turnover, chaperones
- [K] Transcription
- [L] Replication, recombination and repair
- [G] Carbohydrate transport and metabolism
- [D] Cell cycle control, cell division, chromosome partitioning
- [P] Inorganic ion transport and metabolism
- Unknown
- rRNA

159,662 bp
16.5% GC

182 ORFs
98% coding

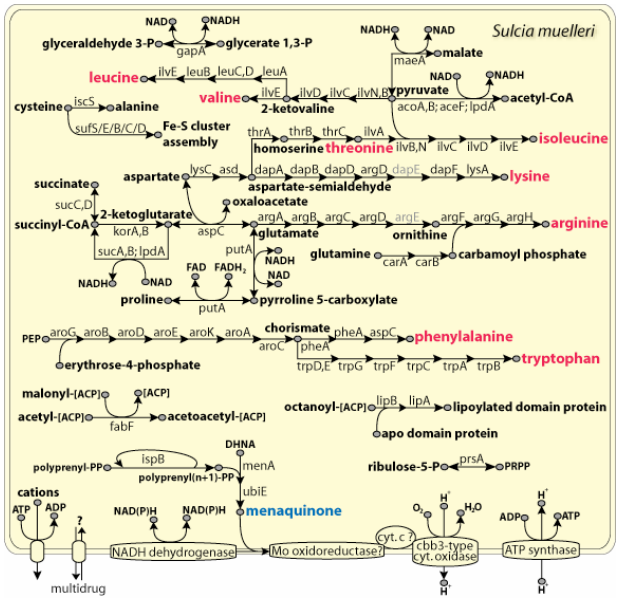
A. Nakabachi, A. Yamashita, H. Toh, H. Ishikawa, H. Dunbar, N. Moran, M. Hattori. 2006. Science 314: 267

Environ Molecular Biology Lab & Human Genome Research RIKEN, JAPAN
School of Science, Kitasato University, JAPAN
Dept. Ecology & Evolutionary Biology, University of Arizona, USA

Genomic properties in *Sulcia* parallel those of other insect symbionts

	Gammaproteobacteria				*Bacteroidetes*		
	E. coli	*Baumannia cicadellin-icola*	*Buchnera* APS	*Carsonella ruddii*	*Bacteroides thetaiota-omicron*	*Porphyr-omonas gingivalis*	*Sulcia muelleri*
Genome size (bp)	4,639,675	686,194	640,681	159,662	6,260,361	2,343,476	245,527
G+C (%)	50.8	33.2	26.3	16.6	42.8	48.3	22.4
Number of genes	4,418	651	607	213	4864	2015	265
Coding density (%)	88.4	88.7	88.9	97.3	89.9	85.2	96.1
Ave CDS length (bp)	954	986	989	826	1174	1014	987

Sulcia makes essential amino acids

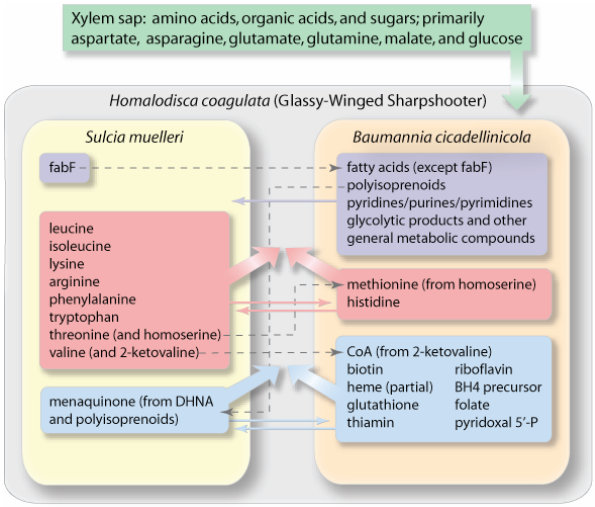


Pathways for all Ess. AA except His, Met (present in Baumannia)

COG-based functional assignment in *Sulcia*:
33.0% translation
21.3% amino acids

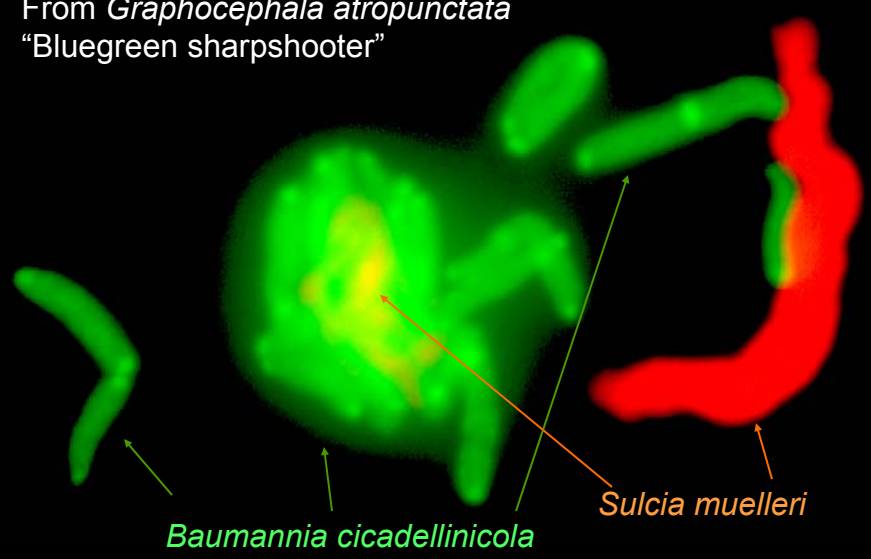
McCutcheon and Moran
PNAS Dec 2007

Metabolic functions of *Baumannia* and *Sulcia* are complementary



McCutcheon and Moran PNAS Dec 2007

From *Graphocephala atropunctata*
"Bluegreen sharpshooter"



P. Tran, U Arizona

Summary

- Sharpshooters contain two unrelated bacterial symbionts within bacteriomes
- These are inherited from mother to offspring
- They are evolutionarily ancient and coevolved with host insects and with one another
- Both symbionts have small genomes that are specialized to provision complementary sets of nutrients rare in the host diet
  - *Sulcia* provides essential amino acids minus histidine
  - *Baumannia* provides vitamins and histidine
  - Nutritional role evident from sequencing genomes of the symbionts

Acknowledgements

University of Arizona: Nicole Gerardo, Helen Dunbar, Howard Ochman ,Phat Tran, Colin Dale, Wendy Smith

Arizona Genomics Institute: Rod Wing, Yeisoo Yu (454 pyrosequencing)

TIGR (now UC-Davis): Jonathan Eisen Dongying Wu (Baumannia genome)
Univ. Illinois: Daniela Takiya Chris Dietrich (leafhopper systematics)
Washington Univ.: Vince Magrini, Matt Hicknbothham, Elaine Mardis (Solexa)
UC-Riverside: Heather Costa (assistance with sampling)

Main Funding: National Science Foundation, Biocomplexity & Microbial Genome Sequencing programs

NIH: postdoctoral support to J. McCutcheon

Center for Insect Science, University of Arizona