# Endosymbiont hunting in the metagenome of Asian citrus psyllid (*Diaphorina citri*)

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# Worldwide distribution of Citrus Greening/Huanglongbing

HLB found in Florida 2005

1st scientific report of HLB in China – 1919

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## West

### Citrus psyllid findings continue in Orange County

06/06/2012 12:14:02 PM
Mike Hornick

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The Asian citrus psyllid has been detected in seven Orange County, Calif., communities this year, but the immediate effect is limited to residential areas.

Between January and May, psyllids were found in Irvine, San Clemente, San Juan Capistrano, Williams Canyon, Ladera Ranch, Mission Viejo and Lake Forest, said Steve Lyle, director of public affairs at th

Orange County has been under quarantine for the psyllid — w disease — since 2009.

The first finding of a diseased tree was in March in a Haciend

*Candi*
*Candi*
*Candi*
*Diaphorina citri*
*Candidatus* Liberibacter africanus, *Candidatus* Liberibacter asiaticus, *Diaphorina citri*, and *Trioza erytreae*
*Diaphorina citri* only

# What is the impact of this disease?

Citrus – a 9.3 billion dollar industry in Florida and 1.2 billion dollars in California

- Leave agricultural practices unchanged - significant loss of production
- Begin expensive , labor intensive controls - increase cost of production




Increased cost of citrus products for consumers

## What is known so far?

**The causal agent:**

All evidence points to the bacterium *Ca.* Liberibacter asiaticus

**How it spreads:**

Vectored by Asian citrus psyllid (ACP)

**How to control it:**

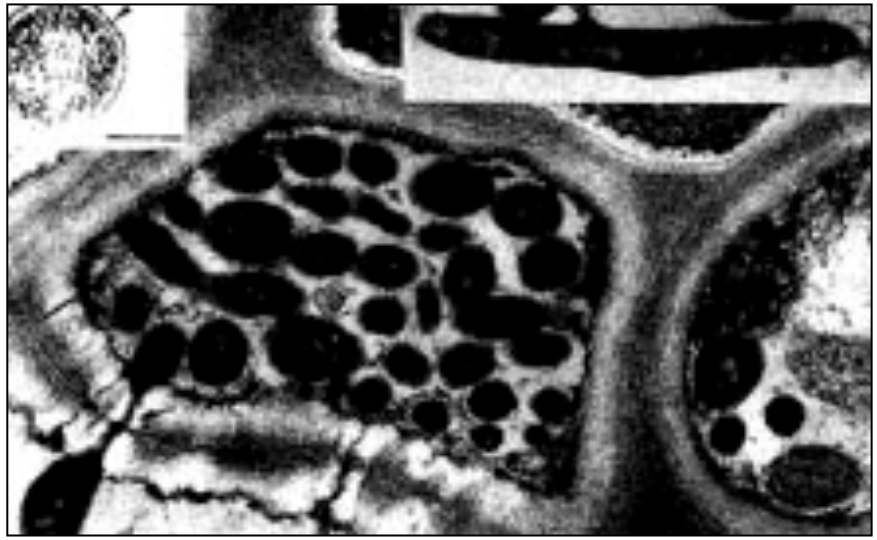
Kill the pathogen
Limit spread of the insect
Limit the spread of infected plants
Resistant host plants

**Complicated by unusual biology**

**The causal agent:**

*Ca.* Liberibacter asiaticus

- 1.2 Mb reduced genome
- Florida strain sequenced



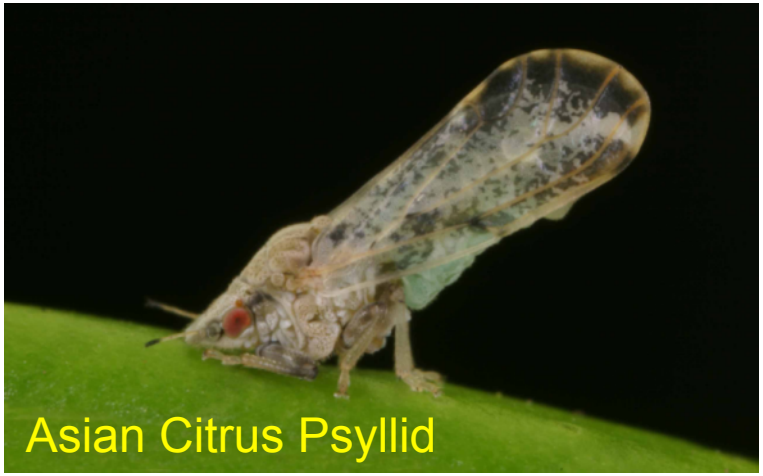
*Ca.* Liberibacter asiaticus in citrus phloem cells

**Complications:**

- Cannot be cultured making experimental characterization very difficult
- Until recently was on the select agent list adding to the difficulties of experimental characterization
- How it causes disease is not clear

## How it gets around:

*Ca.* Liberibacter asiaticus is vectored by




Insect transmission greatly enhances potential for rapid long distance spread

Psyllid feeds on many members of the citrus family

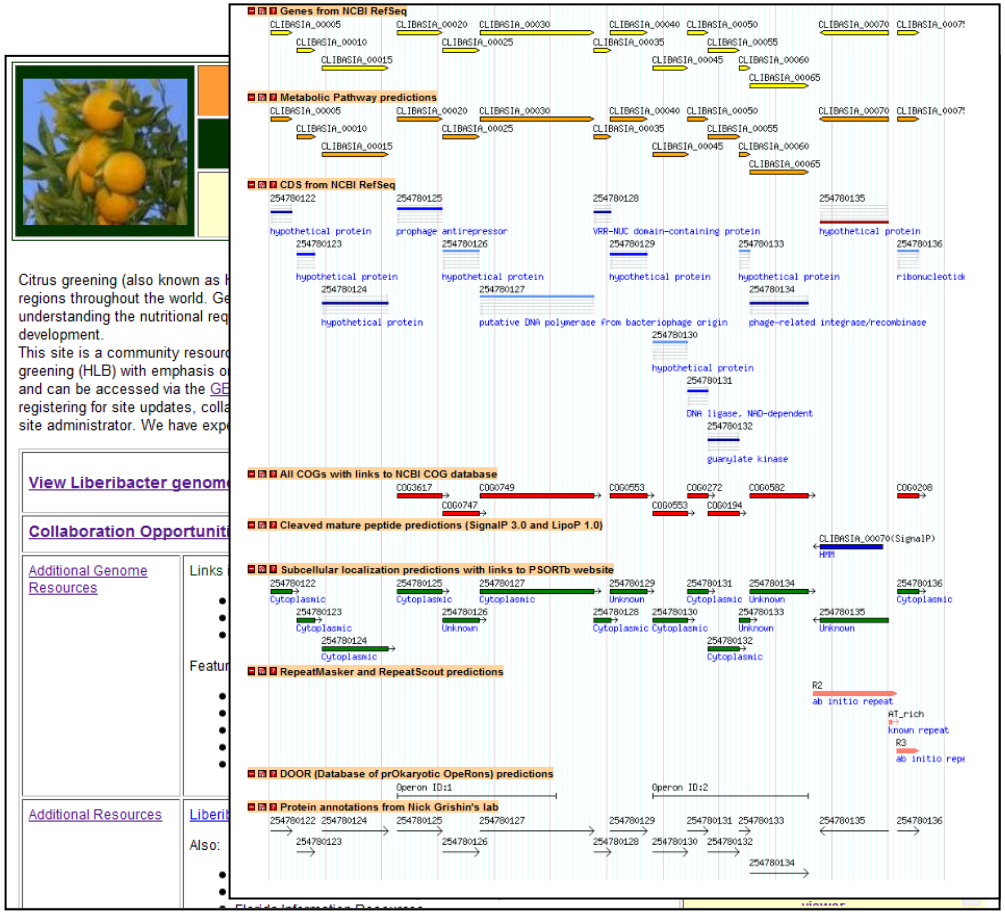
Once infected, each plant becomes a disease reservoir from which uninfected psyllids can pick up bacteria

# Overview

- Citrus Greening website
- *Ca*. Liberibacter asiaticus annotation
- Asian citrus psyllid (ACP)
  - Genome assembly
  - Transcriptome mining for transgenic plants
- Host-endophyte metagenomics
  - Symbionts of ACP
  - Endophytic populations of Maize
- Pseudomonas syringae pathogen genomics
  - T1-like strain
  - Pseudomonas viridiflava

# Citrus Greening website

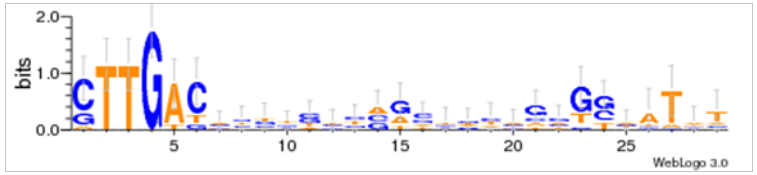
- Community resource
- Genome browser



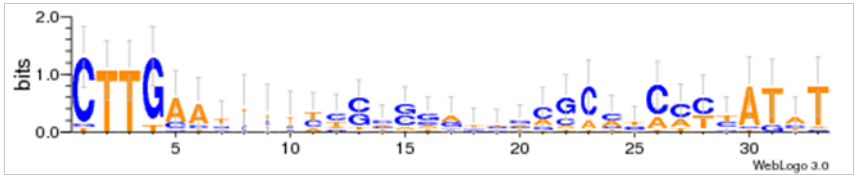

# Genome annotation

- Transcription factors and binding sites
- Subcellular localization of proteins

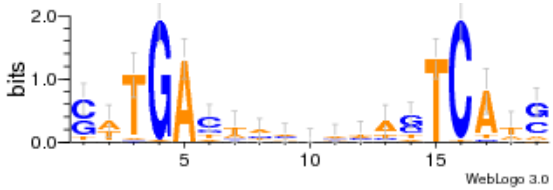
**RpoD** *(primary)*




**RpoH** *(heat)*

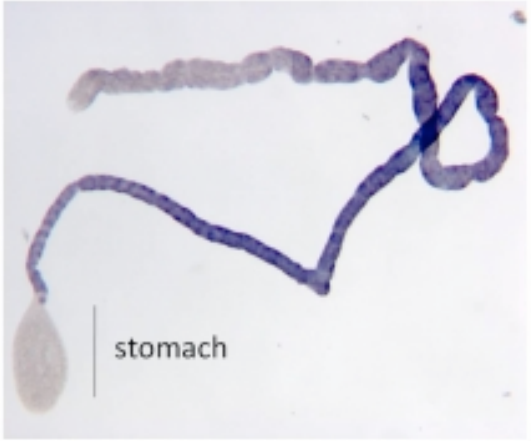



**RirA** *(iron)*



# ACP gene hunting for transgenic plants

- Distrupt the osmotic balance of phloem feeding Hemipterans
- Mining for osmoregulatory genes in ACP
- Targeted denovo transcriptome assembly
  - Potato psyllid
  - White fly
  - Peach-potato aphid
- RNAi-mediated knockdown of osmoregulatory genes

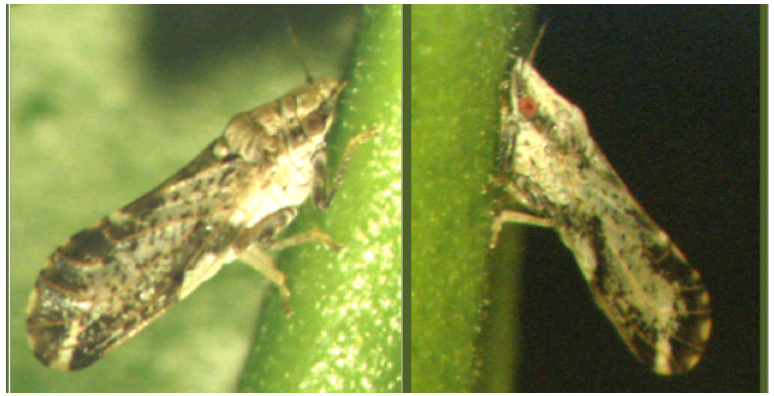



Price 2007

Angela Douglas

# ACP genome assembly

- Sample from Florida
- Illumina sequencing
  - Paired end reads (37 Gb)
  - Mate-pair read libraries
    - 2k (8.7 Gb)
    - 5k (7.5 Gb)
    - 10k (7.1 Gb)
- ~400 Mb genome
- 180X coverage
- Draft assembly analysis
  - Gene finding
  - Repeat identification
- Second round of sequencing with Pacific biosciences underway



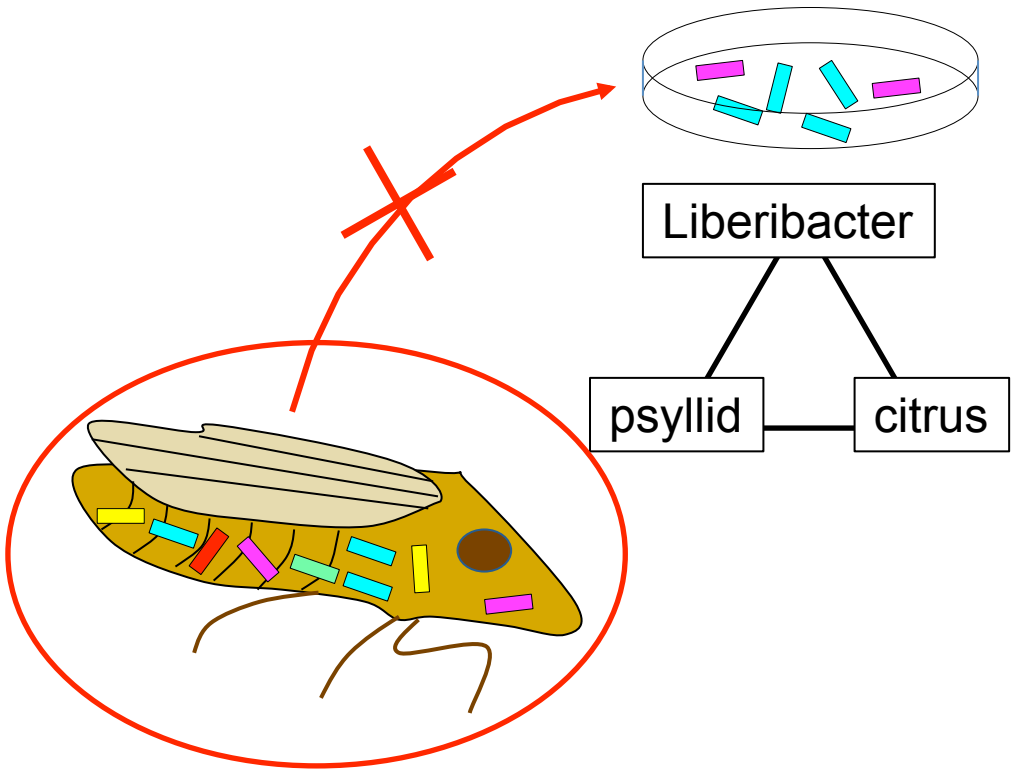
psyllid.org

Wayne Hunter

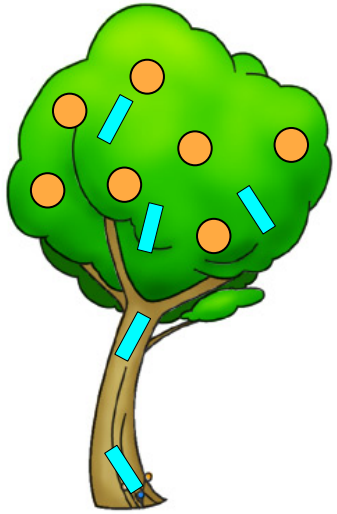


Cliff Han

# Big picture for molecular interactions

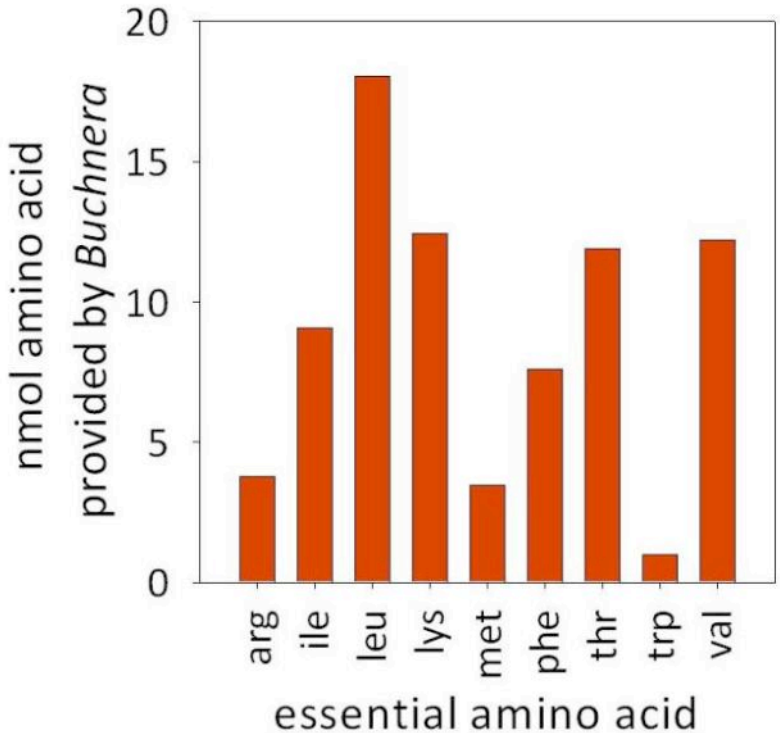



Could lead to identification of metabolically complementary bacteria for co-culturing with Liberibacter



# Why endosymbionts?

- Significant impact on diverse host processes
  - Nutritional status
  - Reproduction
  - Lifespan
  - Resistance to insecticides
- Means to explore population dynamics and relatedness to other isolates
- Known examples of highly-evolved host-symbiont relationship
  - *Buchnera* and Aphids




Buchnera-derived essential amino acids supporting the growth of pea aphid larvae. [ Gündüz and Douglas 2009]

- Psyllid microbiome diversity
  - 10 bacteria reported in ACP (Florida)
  - 5 additional bacteria reported in closely related potato psyllid (*B. cockerelli*)

# Candidate endosymbionts

| Description | Length (bp) | Coverage | Length (bp) |
|---|---|---|---|
| | | | |

# Why Wolbachia?

- Known obligate association with up to 16% of insects species
- Positive correlation of titer with *Ca*. liberibacer asiaticus
- Associated with Cytoplasmic Incompatibility in hosts
- Used for vector control in dengue fever

| | | | |
|---|---|---|---|
| Wolbachia endosymbiont of Culex quinquefasciatus Pel, complete genome | 1,482,455 | 86.787% | 1,140,899 |
| Methylibium petroleiphilum PM1 chromosome, complete genome | 4,044,195 | 0.318% | 20,107 |

# Read capture using baits

- Wolbachia endosymbiont of Drosophila melanogaster
- Wolbachia endosymbiont of Culex quinquefasciatus Pel (wPip)
- Wolbachia sp. wRi
- Wolbachia endosymbiont strain TRS of Brugia malayi

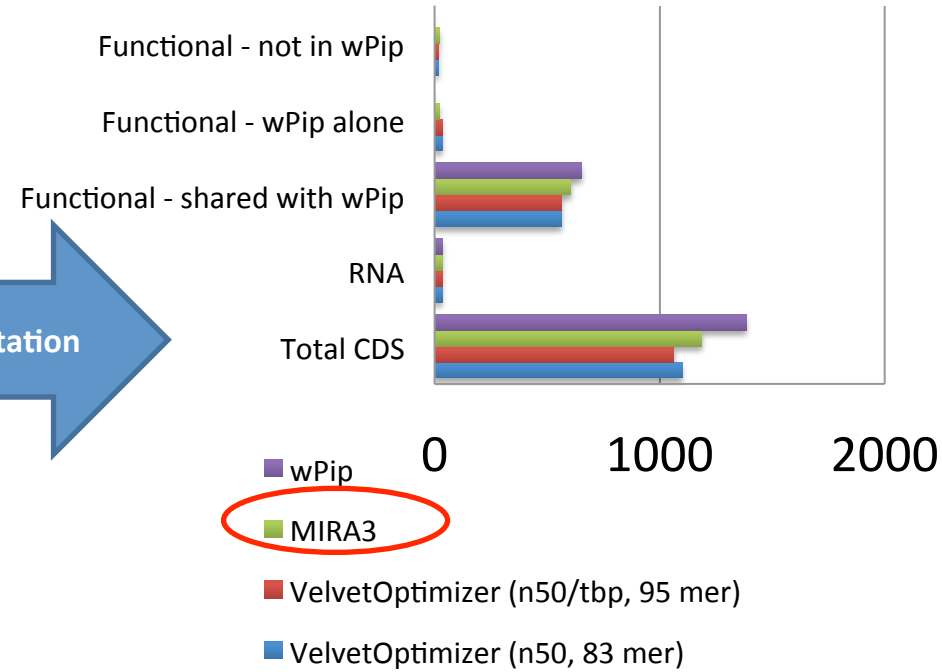
| Dataset | Total (bp) | 100% | 90% | 85% |
|---|---|---|---|---|
| Paired-end | 33,708,218,600 | 127,335,000 | 228,893,000 | 237,906,000 |
| Mate-pair 2k | 7,020,642,800 | 11,200 | 45,200 | 63,000 |
| Mate-pair 5k | 6,000,789,600 | 10,000 | 43,000 | 66,800 |
| Mate-pair 10k | 7,307,423,600 | 2,895,000 | 4,427,600 | 4,909,000 |

Results of mining putative wACP reads from the ACP metagenome

# Workflow

| Assembler | Contigs | N50 | Contigs > 1k | Length of Contigs > 40k |
|---|---|---|---|---|
| VelvetOptimizer (n50, 83 mer) | 111 | 79935 | 53 | 807171 |
| VelvetOptimizer (n50/tbp, 95 mer) | 118 | 84957 | 25 | 744357 |
| MIRA3 | 167 | 23068 | 105 | 247976 |

Annotation

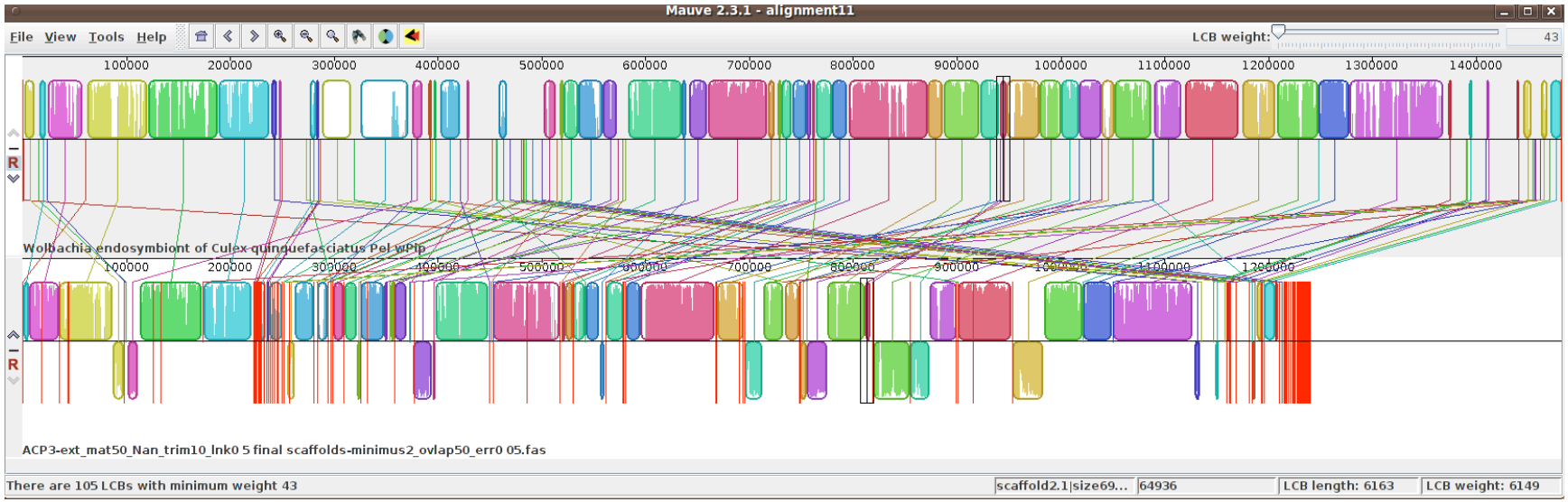



Scaffolding

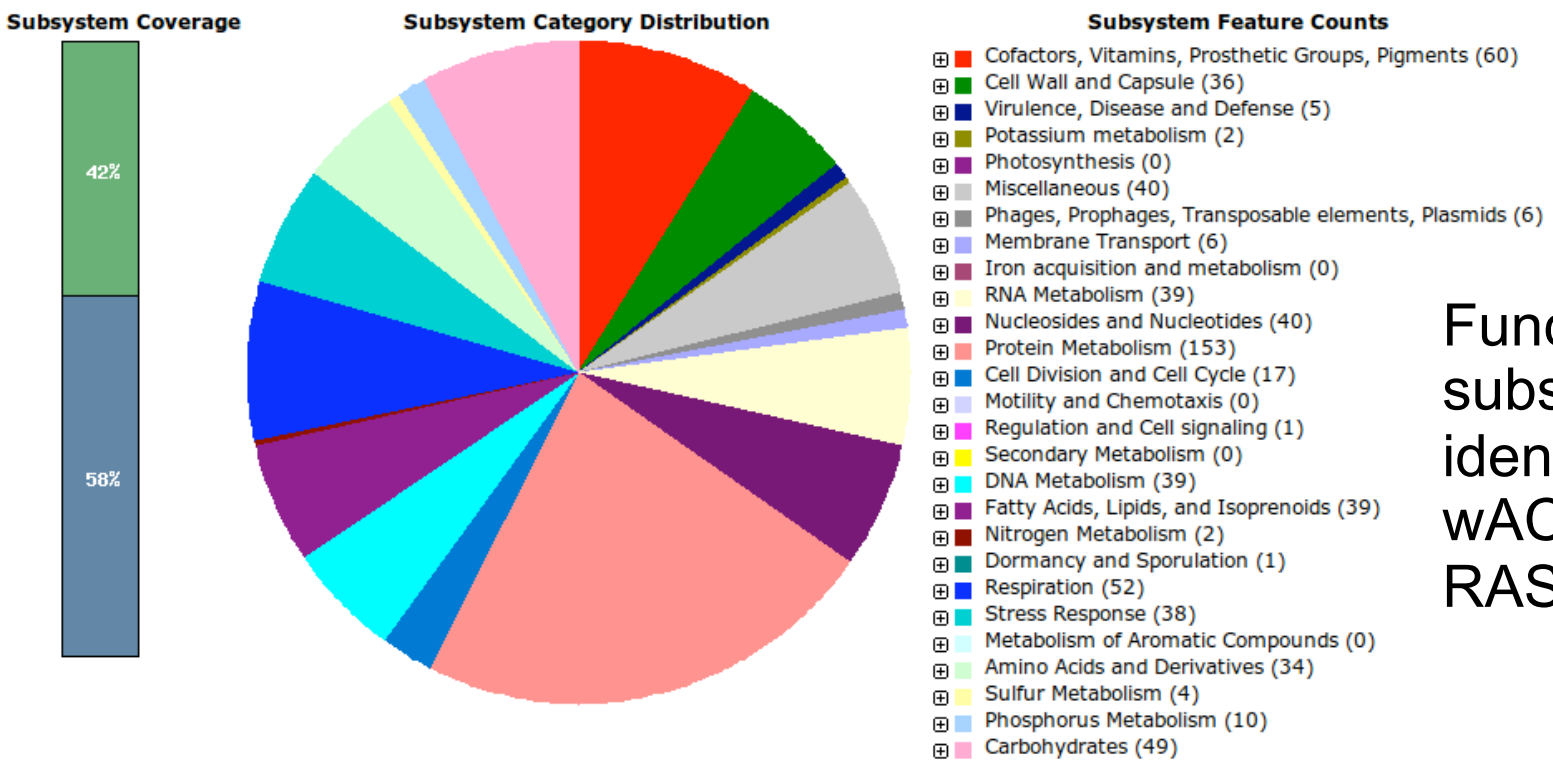
| Description | Contigs | Longest | N50 | Contigs > 1k |
|---|---|---|---|---|
| SOPRA / wACP2 | 101 | 89608 | 41594 | 77 |
| Minimus2 / wACP1 | 99 | 106898 | 41594 | 76 |
| Minimus2 / wACP3 | 104 | 206691 | 52418 | 66 |

Functional annotation using RAST

| | wACP1 | wACP2 | wACP3 |
|---|---|---|---|
| Total CDS | 1176 | 1190 | 1183 |
| RNA | 36 | 36 | 36 |
| Functional - shared with wPip | 608 | 610 | 608 |
| Functional - wPip alone | 24 | 23 | 24 |
| Functional - not in wPip | 23 | 22 | 23 |



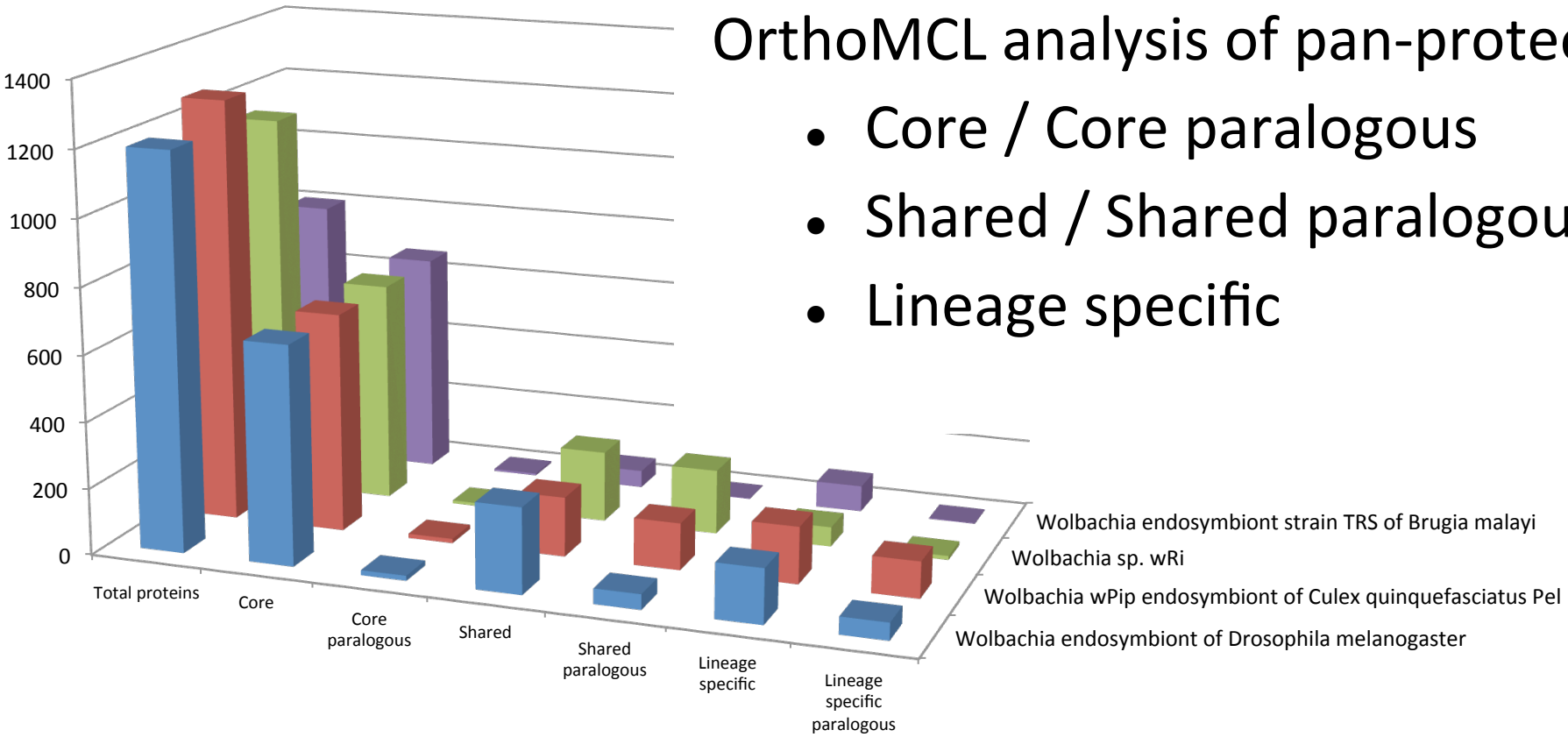

Arranging scaffolds using wPip as reference




Functional subsystems identified in wACP using RAST

# OrthoMCL analysis of pan-proteome

- Core / Core paralogous
- Shared / Shared paralogous
- Lineage specific




- 660 core protein clusters per Wolbachia genome
- 659 core protein cluster representatives found in wACP
- Missing core cluster consists entirely of hypotheticals
- 32 lineage specific genes in wACP w.r.t. wPip
  - All have unknown function
  - 11 have homologs in other endosymbionts of mosquito

**Ankyrin domain proteins**

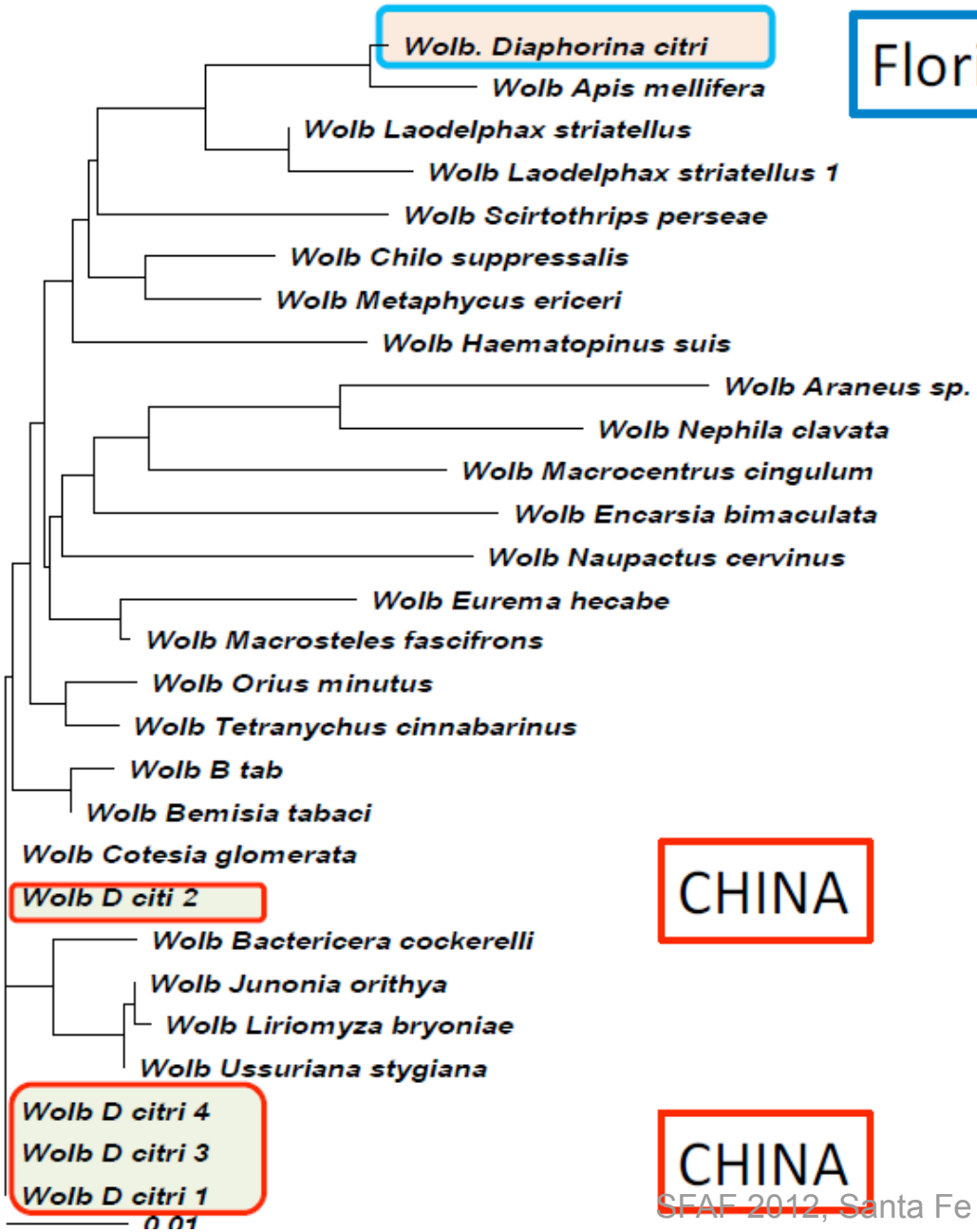
- Tandemly arranged 33 residue repeats
- Sufficiently divergent at nucleotide level to allow assembly
- Candidate for host protein manipulation

- wACP ankyrin repetoire
  - 54 predicted proteins contain ankyrin domain
  - 34 have close homologs in wPip
  - pk2 group of ankyrin proteins present
    - Cytoplasmic incompatibility in Culex
    - Feminization in isopods

**Type IV secretion system**

- 2 cluster arrangement of Type IV genes
- Widely conserved in Wolbachia and present in wACP

# Comparative phylogeny of wACP




Florida, USA

CHINA

CHINA

USA populations formed a separate clade from four wACP populations from China.

# Conclusions

- Targeted genome reconstruction strategy for endosymbionts
- Read mapping approach for candidate endophytes
- Recommendations
  - MIRA3 produced more fragmented but qualitatively better assemblies than Velvet
  - SOPRA and SSPACE are effective for scaffolding when coupled with Minimus2
- Caveats
  - Missing lineage specific regions
  - Low throughput method

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## Asian Citrus Psyllid genome

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- Justin Reese
- Brandi Cantarel

## Pseudomonas pathogens

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- Greg Martin
- Chris Smart
- Lisa Jones

## Asian Citrus Psyllid transcriptomics

- Angela Douglas
- Xiangfeng Jing

## Meta-Maize

- Rebecca Nelson
- Alice Churchill
- Santiago Mideros
- Laura Morales

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CRDF Citrus Research and Development Foundation, Inc.

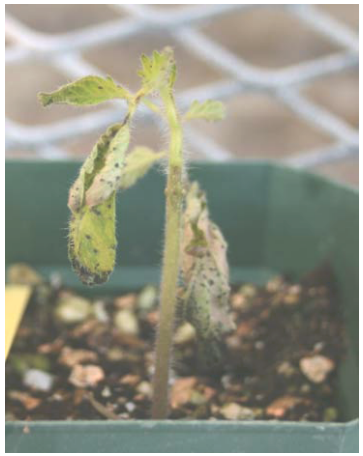
NSF

# Questions??

# Pseudomonas pathogens in NY

## P. syringae pv tomato T1-like strain

- More virulent than T1
- Differences in virulence protein repertoire have been identified
- Mutagenesis assays underway



Pseudomonas syringae pv tomato variant of strain T1 (2009)

## P. viridiflava

Pseudomonas viridiflava (2010)

- Associated with stem rot
- Found to cause leaf spotting on tomato and pepper
- First isolate to be sequenced
- Comparison with other pseudomonad genomes underway

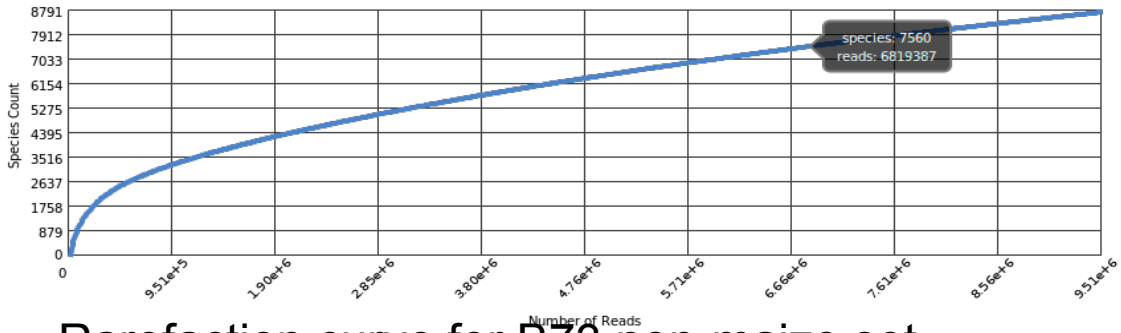
Alan Collmer

# Meta-Maize

## Datasets

- 103 maize inbred lines (Hapmap)
- 10000+ Genotyping by Sequencing maize lines
- 16S and ITS based metagenomics of Nested Association Mapping parents

Host metagenomics using whole genome shotgun reads




Rarefaction curve for B73 non-maize set

## Goals

- Identify core and variable endophytic microbiomes of maize
- Discover associations of endophytic classes with maize genotypes
- Identify variation in endophytic distribution due to
  - Seed source
  - Environmental factors
  - Tissue type

Rebecca Nelson