



A Song of Sap and Blood

CBGP Seminars 2022 - Montpellier, France

Diego Santos-Garcia, Ph.D.

Laboratory of Biometry and Evolutionary Biology
University Lyon 1 - UMR CNRS 5558

February 15th, 2022

Portiera gets wild: Genome instability provides insights into the evolution of both whiteflies and their endosymbionts

Santos-Garcia et al. 2020. Genome Biol. Evol. 12, 2107–2124.



Sternorrhyncha:Aleyrodoidea

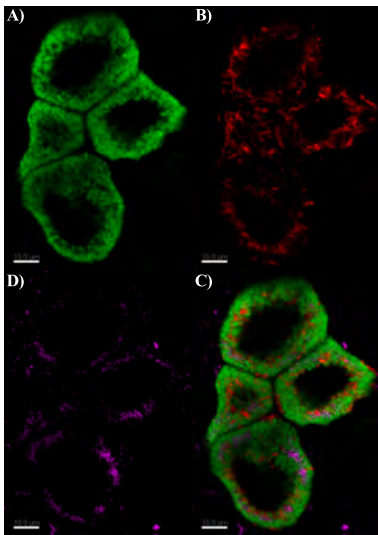
- Paleotropical origin: “the aphids of the tropical regions”.
- Adults are tiny (1-4 mm) and usually covered by wax.
- Four nymphal stages (instar):
 - 1st is the only mobile form - leaf veins hunter.
 - 2nd and 3rd are feeding stages (size increase).
 - 4th or “red eye pupa” is a quiescent stage.



Surendra Dara blog at <http://ucanr.edu/blogs>. 4 th instar photo by Paul de Barro



Whiteflies endosymbionts

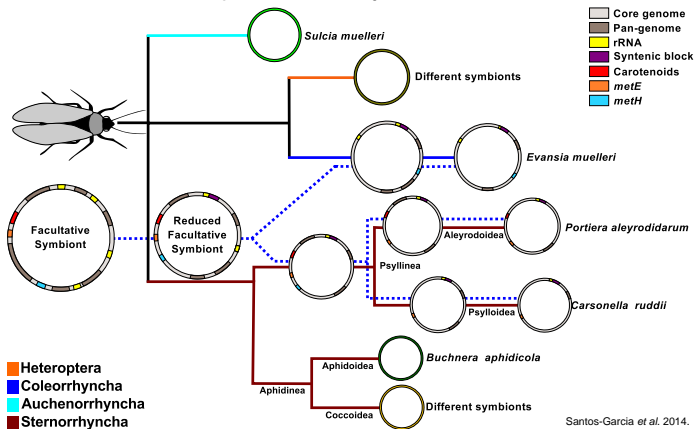


Bemisia tabaci MED nymph's bacteriome (Santos-García 2014).

- Whiteflies always present the P-endosymbiont *Candidatus Portiera aleyrodidarum* (green).
- *Portiera* is devoted to the production of essential amino acids and carotenoids.
- A co-primary, *Hamiltonella* (red) or *Arsenophonus*, supplements B-vitamins to their whitefly host (among other possible benefits).
- Whiteflies may also harbor other secondary endosymbionts: *Wolbachia*, *Rickettsia*, and *Cardinium* (purple).

Portiera, an ancient endosymbiont

- Portiera has **co-diverged** with whiteflies **since their origin** (> 150 Myr).
 - Already a reduced endosymbiont with basic DNA replication, recombination, and repair machinery.

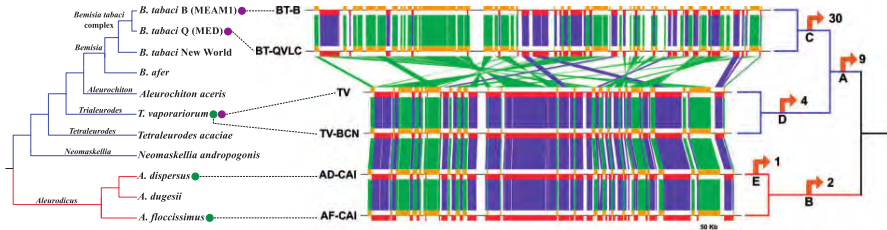


Santos-García et al. 2014.



Portiera, an ancient endosymbiont

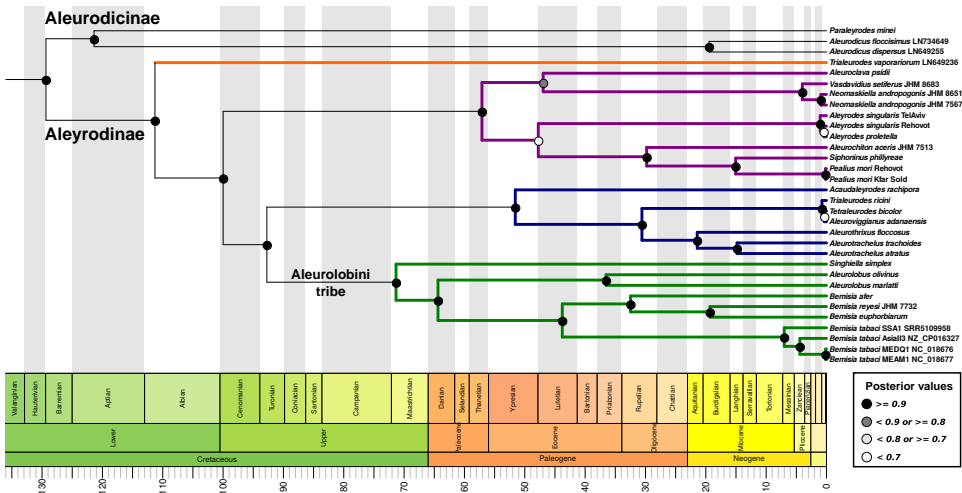
- *Portiera* has **co-diverged** with whiteflies **since their origin** (> 150 Myr).
 - Already a reduced endosymbiont with basic DNA replication, recombination, and repair machinery.
- *Portiera* genomes from most whiteflies are **syntenic** except in *Bemisia tabaci* → **genomic instability**.
- *Portiera* from *B. tabaci* has lost the polymerase proofreading subunit **DnaQ** → cause of instability? (Sloan and Moran, 2013).



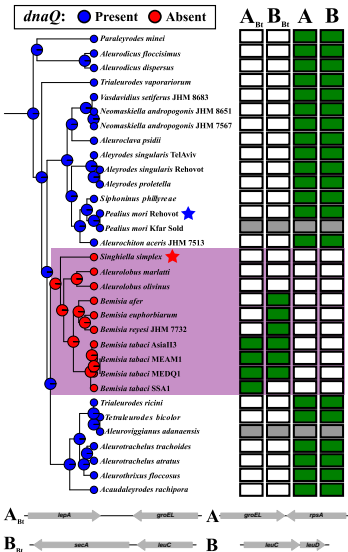
Santos-Garcia et al. 2015



Portiera phylogeny: 16S, 23S, rpoD, dnaK, and groEL



27 whitefly species (19 genera) collected from Israel, La Reunion, Canary Islands, the NHM of London collection, and public databases.

Tracking *Portiera* genome instability

- Screening for the presence of *dnaQ* and different gene order configurations (ancestral or derived).

- *dnaQ* was lost in the *Singhiella*, *Aleurolobus*, and *Bemisia* (**Aleurolobini** tribe) monophyletic clade MRCA (~70 Mya).

- *Bemisia* other than *tabaci* suffered re-arrangements compared to *tabaci*.
- *Singhiella* and *Aleurolobus* do not presented neither the ancestral or *B. tabaci* order.

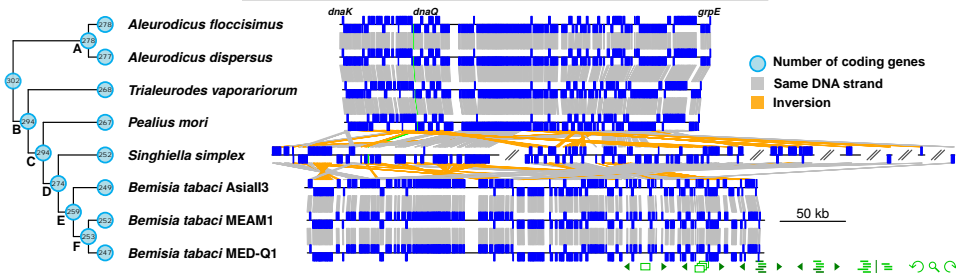
- To confirm the screening results: sequencing *Portiera* from the **most basal** species in the *Bemisia* clade - the fig whitefly *Singhiella simplex*.

- Two *Portiera* with one shot: the sample contained some individuals of *Pealius mori*.



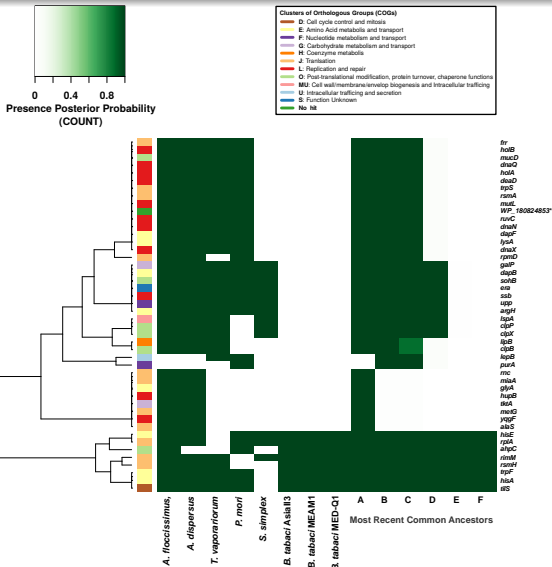
Two new *Portiera* genomes: SiSi and PeMo

	<i>Portiera</i> TeVa	<i>Portiera</i> PeMo	<i>Portiera</i> SiSi	<i>Portiera</i> BeTa
Host	<i>T. vaporariorum</i>	<i>P. mori</i>	<i>S. simplex</i>	<i>B tabaci</i> MED-Q1
Genome size (bp)	280822	277700	411975	357472
Contigs	1	1	9	1
Genes*	307	308	300	284
CDS	268	266	252	247
Pseudogenes (CDS)	1	3	11	7
Intergenic avg. length	62.79	51.81	715.44	524.99
Coding Density (%)	91.46	96.60	59.56	69.60
Intergenic regions (%)	8.54	3.40	40.44	30.40
rRNA	3	3	3	3
tRNA	34	34	34	33
tmRNA	1	1	1	1
RnaseP RNA	1	1	1	1
<i>dnaQ</i>	yes	yes	pseudo	no
Direct repeats	1	2	23	4
Inverted repeats	0	1	17	2
Tandem repeats	10	31	3	111



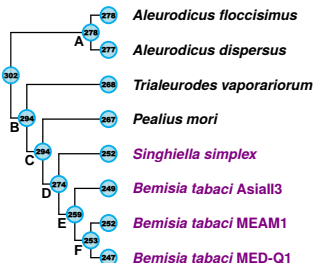


Portiera gene losses history



Species/MRCA	Gene name
<i>A. dispersus</i> (AIDi)	<i>ahpC</i>
<i>T. vaporariorum</i> (TeVa)	<i>rpmD, purA, ahpC, hisE, rplA</i>
<i>P. mori</i> (PeMo)	<i>clpP, clpX, lspA, rimM, rsmH</i>
<i>S. simplex</i> (SiSi)	<i>rsmH, ahpC, hisA, tilS, trpF</i>
A	<i>purA, lepB</i>
B	<i>glyA, hupB, metG, miaA, mrc, tktA, yqgF, alaS</i>
C	<i>clpB, lipB</i>
D	<i>purA, lepB, rpmD, dapF, deaD, dnaN*, dnaQ*, dnaX*, fir, holA*, holB*, lysA, mucD, mutL*, rsmA, ruvC*, trpS, hypothetical protein</i>
E/F	<i>clpP, clpX, lspA, argH, dapB, era, galP, sohB, sssB*, upp</i>

*DNA replication and repair.





Take home message

- **Genome instability is rare in P-endosymbionts** and could point to interesting evolutionary events in both the symbiont and the host.
- A **massive DNA replication and repair genes loss** happen **in** the MRCA of the **Aleurolobini** tribe and is **linked to Portiera genome instability**.
- Instability is not exclusive of *B. tabaci* species: no role on diversification.
- **Single-bacteriocyte inheritance** could have **decreased Portiera's N_e** , making purifying selection unable to maintain the DNA replication/repair machinery.
- Instability first → **host compensatory adaptation** to increase purifying selection each generation by **eliminating defective Portiera** populations.
- *B. tabaci* has acquired multiple bacterial genes to compensate for *Portiera* losses compared to *T. vaporariorum* (Bao et al., 2021; Ren et al., 2020, 2021):
 - Consequence of single-bacteriocyte inheritance or *Portiera* genome degradation?

Acknowledgments



In memory of Prof. Dan Gerling
(1936–2016)



Shai Morin, Natividad Mestre-Rincon

Einat Zchori-Fein



David Ouvrard

Whitefly Samples: Dra. Estrella Hernández Suárez and Dr. Hélène Delate

Fundings:



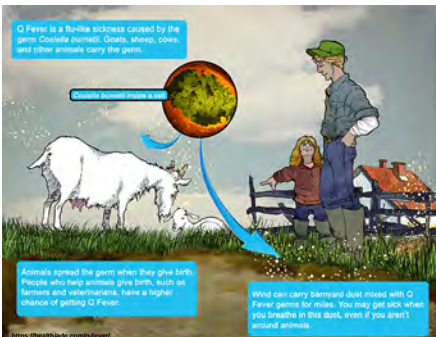
Postdoctoral Fellowships: Valazzi Pikovski Fund and The Golda Meir Fellowship Trust



Comparative genomics sheds new light on the evolution of pathogenicity in *Coxiella burnetii*.

Santos-Garcia et al. 2022. Unpublished.

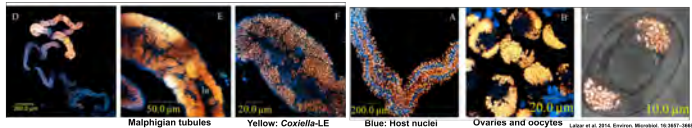
Coxiellaceae family: *Coxiella burnetii* & Q-fever



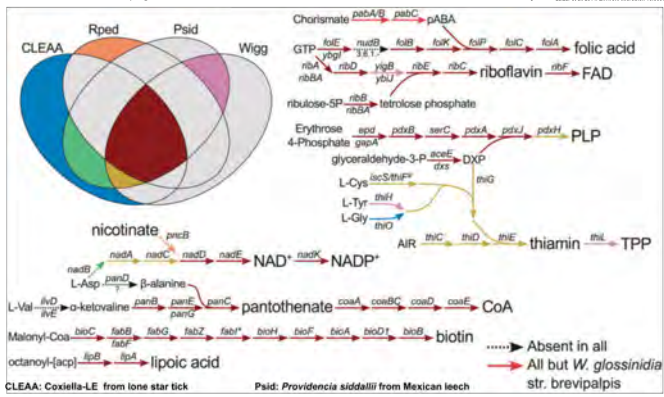
- The **Coxiellaceae** family (γ -proteobacteria) is composed of **five genera** showing lifestyles ranging from **free-living to mutualistic**.
- ***Coxiella burnetii*** is an **intracellular parasite** with a **biphasic life cycle**.

- The Small Cell Variant (**SCV**) is a resistance form (\sim **spore**), persisting in the environment for extended periods \rightarrow **infective** form.
- SCV is activated by the acid pH of the host cell phagolysosome \rightarrow Large Cell Variant (**LCV**) \rightarrow **replicative** and metabolically active **form**.

The good relatives: *Coxiella*-like endosymbionts



- *C. burnetii* relatives have been found associated with ticks.



CLEAA: *Coxiella*-LE from lone star tick
 Rped: *Riesia pediculicola* from human body louse
 Psid: *Providencia siddallii* from Mexican leech
 Wigg: *Wigglesworthia* sp. from Tsetse flies

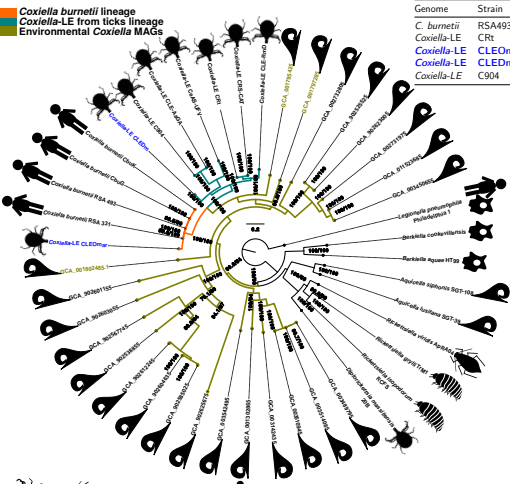
Manzano-Marin et al. 2015. Genome Biol. Evol. 7:2871-2884

- **Coxiella-like endosymbionts** (*Coxiella*-LEs or CLEs) **supply** their tick hosts with **B-vitamins** scarce in blood-based diets.
- Are *Coxiella*-LEs domesticated pathogens?



Coxiella relatives and two new Coxiella-LEs

Coxiella burnetii lineage
Coxiella-LE from ticks lineage
Environmental Coxiella MAGs



Main Host/Environment

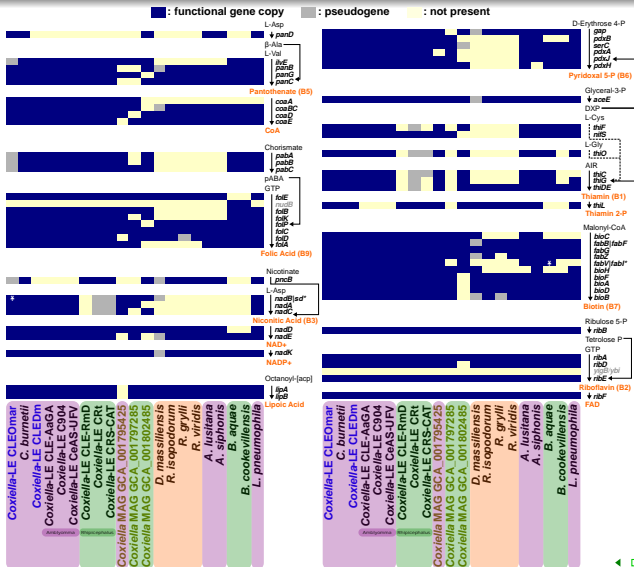
Maximum Likelihood phylogenomic tree of Coxiellaceae genomes inferred from a concatenated alignment of 143 core proteins.

Genome	Strain	Host	Size (Mb)	CDS	Pseudogenes	IS types/copies	%GC	Contigs
<i>C. burnetii</i>	RSA493	Mammals/Amoebas	1.99	1833	207	6/32	42.6	1
<i>Coxiella-LE</i>	Crt	<i>Rhipicephalus turanicus</i>	1.73	1293	337	0/0	38.2	1
<i>Coxiella-LE</i>	CLEOmar	<i>Ornithodoros maritimus</i>	1.83	976	608	4/31	41.5	112
<i>Coxiella-LE</i>	CLEDm	<i>Dermacentor marginatus</i>	0.9	658	15	0/0	35.1	1
<i>Coxiella-LE</i>	C904	<i>Amblyomma americanum</i>	0.66	565	3	0/0	34.6	1

- CLEDm is an old endosymbiont: small genome, low GC, no mobile elements.
- CLEOmar is a recent endosymbiont: reduced genome with pseudogenes, mobile elements, and high GC.
- *C. burnetii*, *Coxiella*-LEs, and environmental *Coxiella* MAGs form a monophyletic clade.
- *C. burnetii*/*Coxiella*-LEs clade originated from aquatic bacteria able to establish symbiotic relationships.

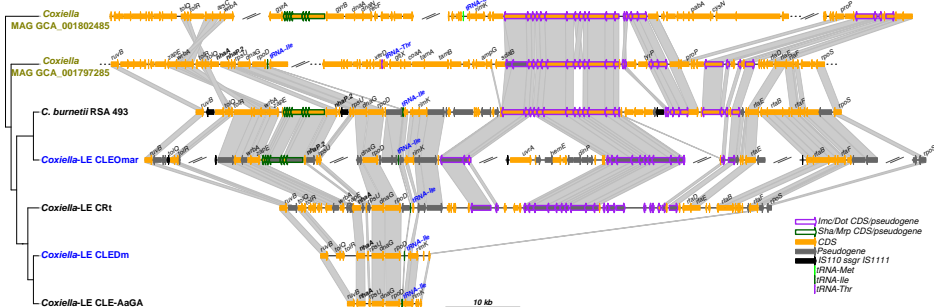


Coxiellaceae biosynthetic potential

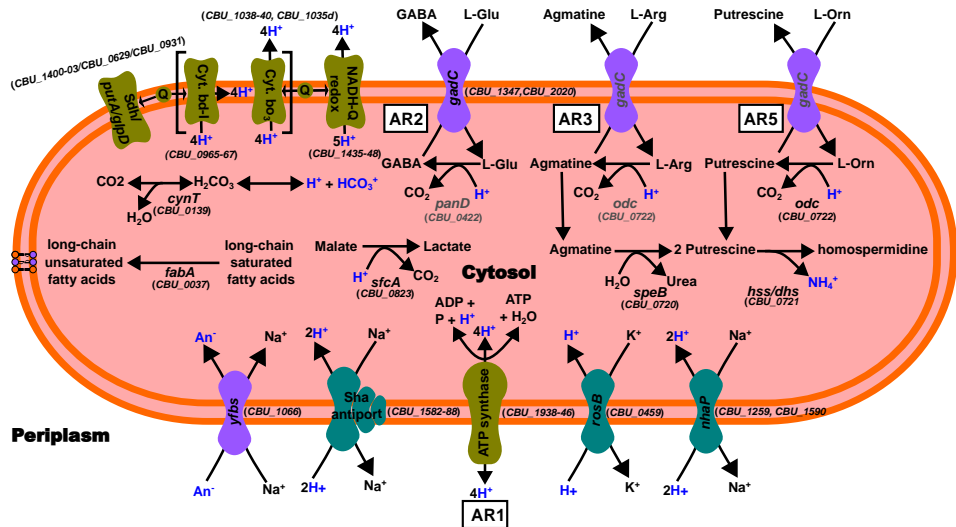


- Coxiellaceae have different biosynthetic potential (~three groups).
- Coxiellaceae can produce riboflavin (B2) and lipoic acid.
- *Coxiella* representatives produce all B vitamins.
- *Coxiella* pre-adaptation to mutualism with hematophagous arthropods?

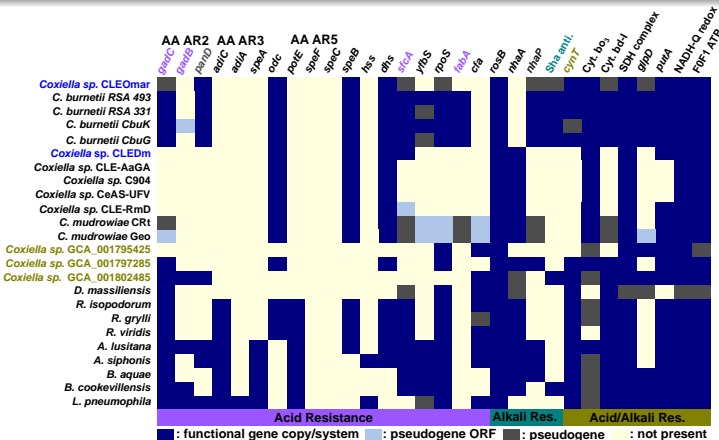
The Dot/Icm T4 Secretion System in Coxiellaceae



- The **Dot/Icm SS** is present in all Coxiellaceae except in reduced *Coxiella*-LEs: an **ancestral trait for host cell invasion and manipulation** (Brenner et al., 2021)
 - **SSs losses** are expected during evolution **towards mutualism** (Oakeson et al., 2014).
 - In *C. burnetii*, the **Dot/Icm SS** region presents signatures of **pathogenic islands (PAIs)**: tRNAs, IS elements, DR, HT genes, and virulence factors (Hacker and Kaper, 2000).
 - *C. burnetii*'s **PAI acquired** additional genes (a **Sha/Mrp antiporter**) while in *Coxiella*-LEs is losing genes related to pathogenesis.

pH Homeostasis in *C. burnetii*

pH Homeostasis in Coxiellaceae



- *C. burnetii* potentially use three AA ARS.
- **Coxiella-LEs** are harbored in non-acidic host-derived vacuoles and **ARS are not required** (Brenner et al., 2021)

- **Sha/Mrp antiporter** is almost exclusive to *C. burnetii*'s lineage.
- Involved in alkali resistance, establishment of symbiotic interactions, and **sporulation** (Ito et al., 2001)

Take home message

- *Coxiella* ancestor was an **environmental facultative symbiont able to invade different hosts**, thanks to the presence of the Dot/Icm T4SS and the ability to deal with abiotic stress.
- This ancestor splitted in **two lineages**, one including *C. burnetii* and the other mainly **tick-associated symbionts**.
- **All Coxiella-LEs produce B vitamins**, which seems the reason for their mutualistic symbiosis with ticks (Duron and Gottlieb, 2020).
 - The potential to **synthesize vitamins is required for initiating mutualistic symbiosis** in ticks.
- The **Icm/Dot T4SS** is part of the same **pathogenic island** present in *C. burnetii* and *Coxiella*-LEs, although reduced in the last.
- The **Sha operon may be related to the SCV morphotype** of *C. burnetii* and their ability **to resist** alkaline environments found **outside the host**: future research on anti Q fever strategies?

Acknowledgments

Fabrice Vavre



Olivier Morel



Olivier Duron



Valérie Noël



Karen McCoy



Lionel Zenner



H el ene Henri



Adil El Filali

ANR hMICMAC: Host-Microbiota Co-Adaptations - Mechanisms and consequences.

Projets Exploratoires Premier Soutien (PEPS) Interdisciplinaires



Koret School of Veterinary Medicine



Yuval Gottlieb

UPPSALA UNIVERSITET



Lisa Klasson

Disentangling the multitrophic interactions of the supervector *Bemisia tabaci* to potentially use its symbiotic communities to reduce plants viral-vectorated diseases.

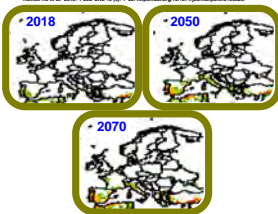
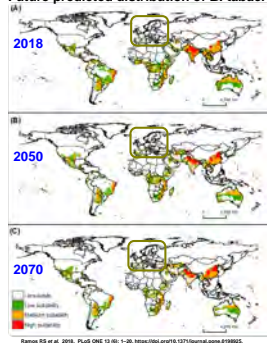
GuardSym MSCA-IF Grant agreement ID 885583

“The FAO estimates that between **20 % and 40 % of world agricultural production is lost each year due to pests.** Plant diseases cost the world economy **\$220 billion annually,** and invasive insects \$70 billion (www.fao.org).”

“Around **47 % of plant diseases are caused by viruses,** most of them vectored by insects (Navas-Castillo *et al.* 2011).”

Intensive agriculture and Global warming

Future predicted distribution of *B. tabaci*



- Intensive agriculture: **biodiversity and beneficial insects reduction.**
 - Monocultures and irrigation.
 - Introduction of non-native crops.
 - Chemical abuse: fertilizers, herbicides, and insecticides.
- Global warming: **expansion of pests insects and vectored diseases.**
 - Increased temperatures, changes in rainfall patterns and desertification.
- Most **phyto-viruses require a vector** to infect new plants:
 - **Sap sucking insects**, such as whiteflies, are the most **efficient** vectors.

Whiteflies: a supervector



- Feeds on more than **600 plants**, including crops such as:

- **Cassava** (Malpighiales) -> Staple food in Africa.
- Potato, sweet-potato, tomato, pepper, aubergine... (Solanaceae).
- Pumpkin, zucchini, melon... (Cucurbitaceae).
- Beans, broad beans, soybeans, alfalfa, peanuts... (Legumes).
- Cauliflower, broccoli, cabbage... (Brassicaceae).

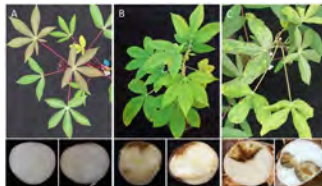
Whiteflies: a supervector

Navas-Castillo J et al. 2011. Annu. Rev. Phytopathol. 49:219–248.



The University of Georgia College of Agricultural and Environmental Sciences

- Develops **resistance to pesticides**.
- Transmits more than **300 phyto-viruses**: global pandemic in tomato (TYLCV) and cassava (CSBD and ACMV).
- Need for Integrated Pest Management (IPM): set of strategies and practices that respect the environment, **minimizing the use of pesticides**.



Sheat S et al. 2019. Front. Plant Sci. 10:567.



Mosquitoes, dengue, and population replacement (IPM)

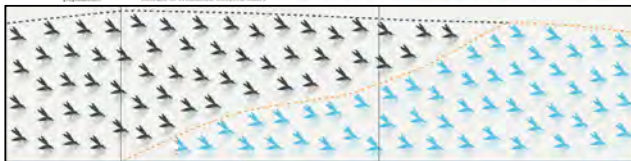


Image by O'Neill S.



- In Queensland (Australia) it has been possible to **eliminate dengue** since 2011 using *Wolbachia* (299Km²).
- *Wolbachia* is an intracellular bacterium present in >50 % of insects.

Wild mosquito population Release period: Release of Wolbachia-infected males Mosquitoes without Wolbachia Mosquitoes infected with Wolbachia



Modified from Flores HA & O'Neill S. 2018. Nat. Rev Micro 18:508-518.

- *Wolbachia* is able to **invade natural mosquito populations** by manipulating their reproduction (Cytoplasmic Incompatibility or CI).
- *Wolbachia*-infected mosquitoes do not transmit dengue, Zika, chikungunya, and yellow fever **viruses** (Heather & O'Neill 2018).

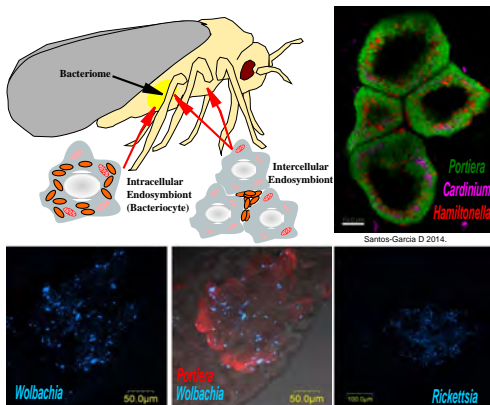
The Guardian Symbionts (GuardSym) project focuses on identifying **symbiotic bacteria** naturally present in **whiteflies** that may confer **viral resistance**.

GuardSym tries to lay the foundations for the **development of population modification strategies** in which natural populations are replaced by others unable to vector viral diseases.

Insects artificially infected with natural strains of *Wolbachia* (or other **symbiotic bacteria**) are not considered Genetically Modified Organisms - **permitted use** in the European Union.

Symbiotic bacteria in whiteflies

- All whiteflies host **various symbiotic bacteria**.
- Bacteriocyte: cells specialized in harboring symbiotic bacteria.
- *Portiera* is essential to the insect and is always present.

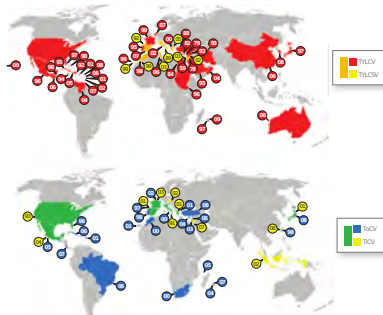


Santos-Garcia D 2014.

Gottlieb Y et al. 2008. FASEB J. 22: 2591-2599.

GuardSym: Basic Objectives

- Study insect-vectored viral diseases as a set of **multitrophic interactions** between insects, bacteria, viruses, and plants.
- Establish the whitefly *B. tabaci*, one of the worst invasive species and present in Europe, as a viral vector model.
- Unravel multitrophic interactions in two of the most widely distributed tomato model viruses: TYLCV (Begomovirus) and ToCV (Crimivirus).

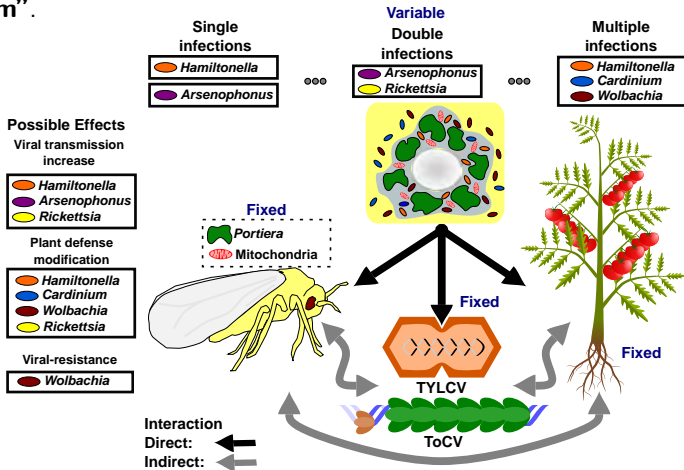


Supervector	Genus	Properties		Mode of transmission	Number of species ^a
		Virion shape and size	Genetic material		
<i>Bemisia tabaci</i> (sweet potato/obacco/silverleaf whitefly)	<i>Begomovirus</i>	Geminate, 18 × 30 nm	ssDNA	Persistent circulative	288
	<i>Ipomovirus</i>	Flexuous, filamentous, 12–15 × 800–950 nm	ssRNA	Semipersistent	6
	<i>Crimivirus</i>	Flexuous, filamentous, 10–13 × 650–900 nm	ssRNA	Semipersistent	13
	<i>Carlavirus</i>	Flexuous, filamentous, 12–13 × 470–1,000 nm	ssRNA	Semipersistent	2 (of 52) ^b
	<i>Torradorvirus</i>	Spherical, 28–30 nm	ssRNA	Semipersistent	2 (3) ^c

Gilbertson RL et al. 2015. Annu. Rev. Virol. 2:67–93.

GuardSym: Applied Objectives

- Identify useful symbiotic bacteria, or their combinations, for IPM strategies in whiteflies and other sucking insects by fixing the rest of “variables” in the “system”.



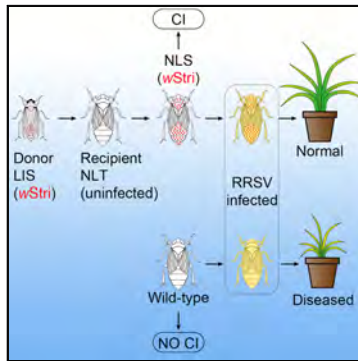
Use of symbionts in other agricultural pests

Article

Current Biology

Stable Introduction of Plant-Virus-Inhibiting *Wolbachia* into Planthoppers for Rice Protection

Graphical Abstract



Authors

J un-Tao Gong, Yongjun Li,
Tong-Pu Li, ..., Xiao-Yue Hong,
Ary A. Hoffmann, Zhiyong Xi

Correspondence

xyhong@njau.edu.cn (X.-Y.H.),
ary@unimelb.edu.au (A.A.H.),
xizy@msu.edu (Z.X.)

In Brief

Gong et al. report the first successful transfer of a *Wolbachia* endosymbiont into a pest planthopper. The endosymbiont self-spreads into host populations and inhibits transmission of a rice plant virus by *Nilaparvata lugens*. The findings open the way for a new approach to limiting crop damage caused by agricultural pests.



**European Union's Horizon 2020 - Marie Skłodowska-Curie
Grant Agreement No 885583**



That's all Folks!



HAVE FUN!

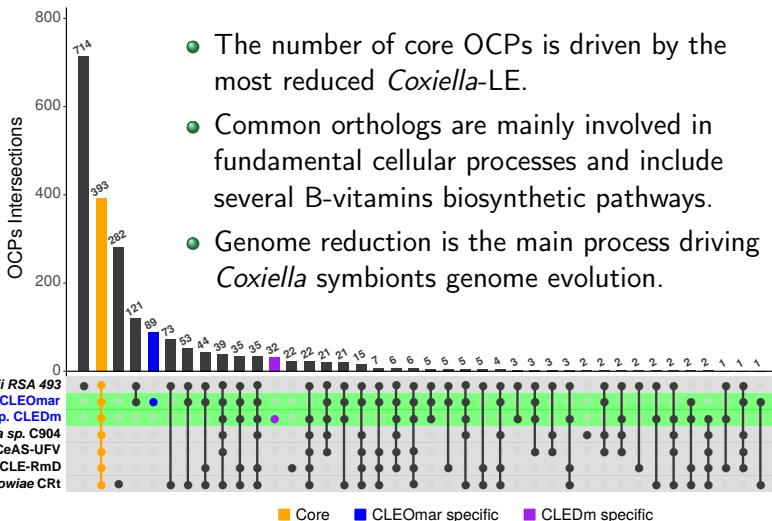


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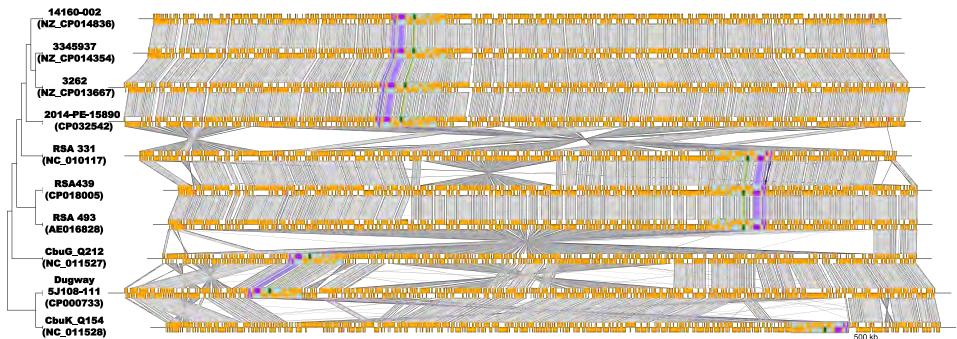
- 1 *Portiera* gets wild: Genome instability provides insights into the evolution of both whiteflies and their endosymbionts
 - Aleyrodidae superfamily or whiteflies
 - Whiteflies endosymbionts
 - Whiteflies-*Portiera* co-evolution
- 2 Comparative genomics sheds new light on the evolution of pathogenicity in *Coxiella burnetii*.
 - The Coxiellaceae family
 - Coxiellaceae genome evolution
 - Pathogenesis evolution & pH homeostasis
- 3 Disentangling the multitrophic interactions of the supervector *Bemisia tabaci* to potentially use its symbiotic communities to reduce plants viral-vectorred diseases.
 - Viral emergent diseases
 - Whiteflies: a supervector
 - Guardian Symbionts MSCA

C. burnetii & *Coxiella*-LE OCPs

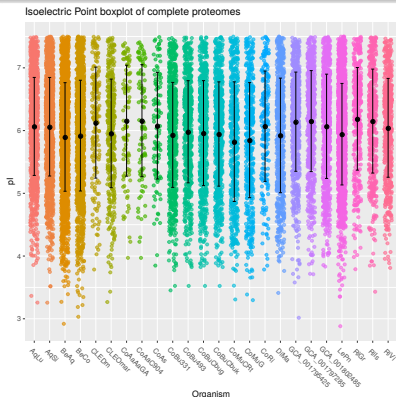
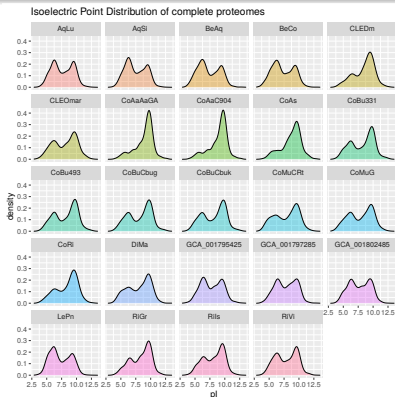


- The number of core OCPs is driven by the most reduced *Coxiella*-LE.
- Common orthologs are mainly involved in fundamental cellular processes and include several B-vitamins biosynthetic pathways.
- Genome reduction is the main process driving *Coxiella* symbionts genome evolution.

PAI syntenry in *C. burnetii*



pH homeostasis: protein buffering



- Acid-resistance may be conferred by basic proteins - “proton” buffering.
- *C. burnetii* encodes large number of basic proteins (average pI \sim 8.25) compared to other bacterial sequenced genomes (e.g. *Helicobacter pylori*).
- Average pI values and distribution are not so different from other *Coxiellaceae*.

Guardian Symbionts: introgression

