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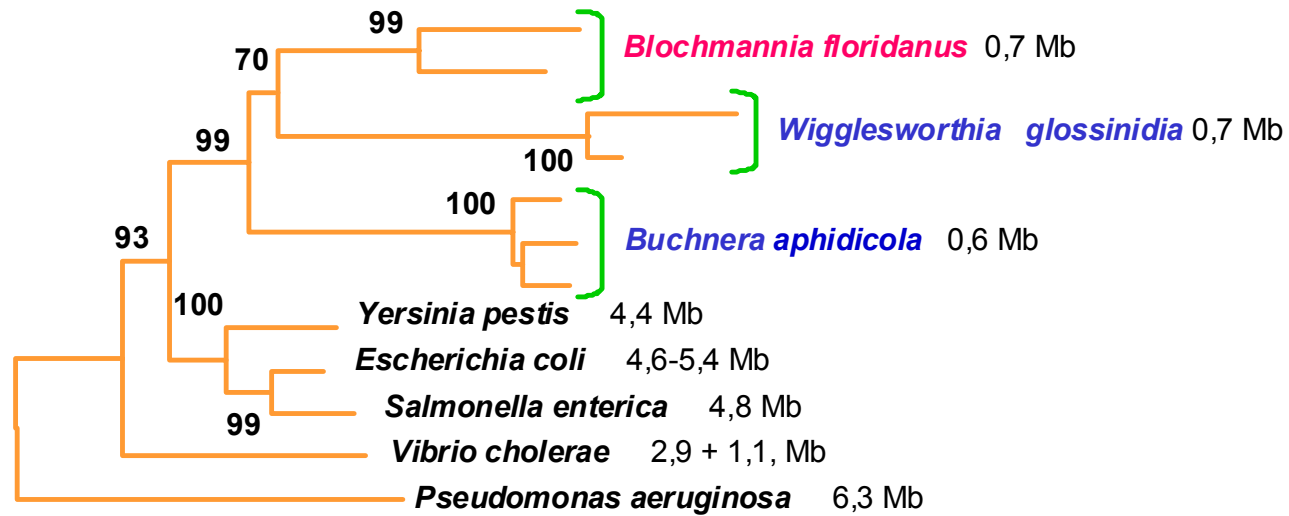
Caracterización del genoma completo de *Blochmannia floridanus*,

bacteria endosimbionte primario
de la hormiga carpintero

Dra. Rosario Gil
Paterna, 2003



Simbiosis bacteria-insecto



Camponotus floridanus



Glossina palpalis



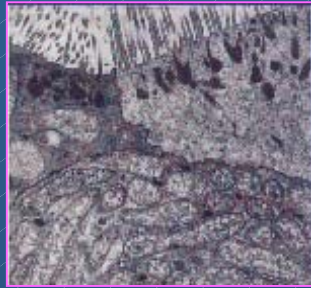
Acyrthosiphon pisum



Blochmannia spp.

➔ *Camponotus* sp. y *Blochmannia* sp.:

- la simbiosis bacteria-insecto más antigua conocida (Blochmann, 1892)



- Subfamilia Formicinae (70 MY)
- Relevancia de la simbiosis: primeras fases de la vida del insecto
- ¿Función biológica?

* *Camponotus* sp. pueden alimentarse con una dieta compleja



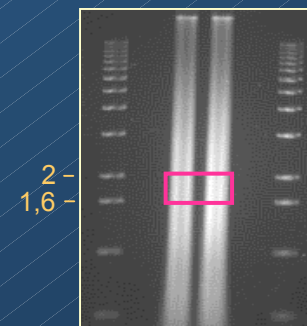
Proyecto genómico



Camponotus floridanus (100 pupas)



DNA *Blochmannia floridanus* (97% puro)



Genoteca "shotgun" (tamaño medio de inserto: 1,5 Kb)



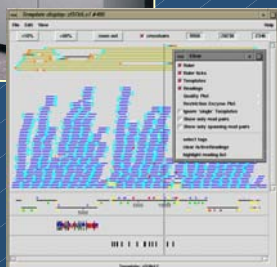
Purificación plásmidos (6.528 clones)

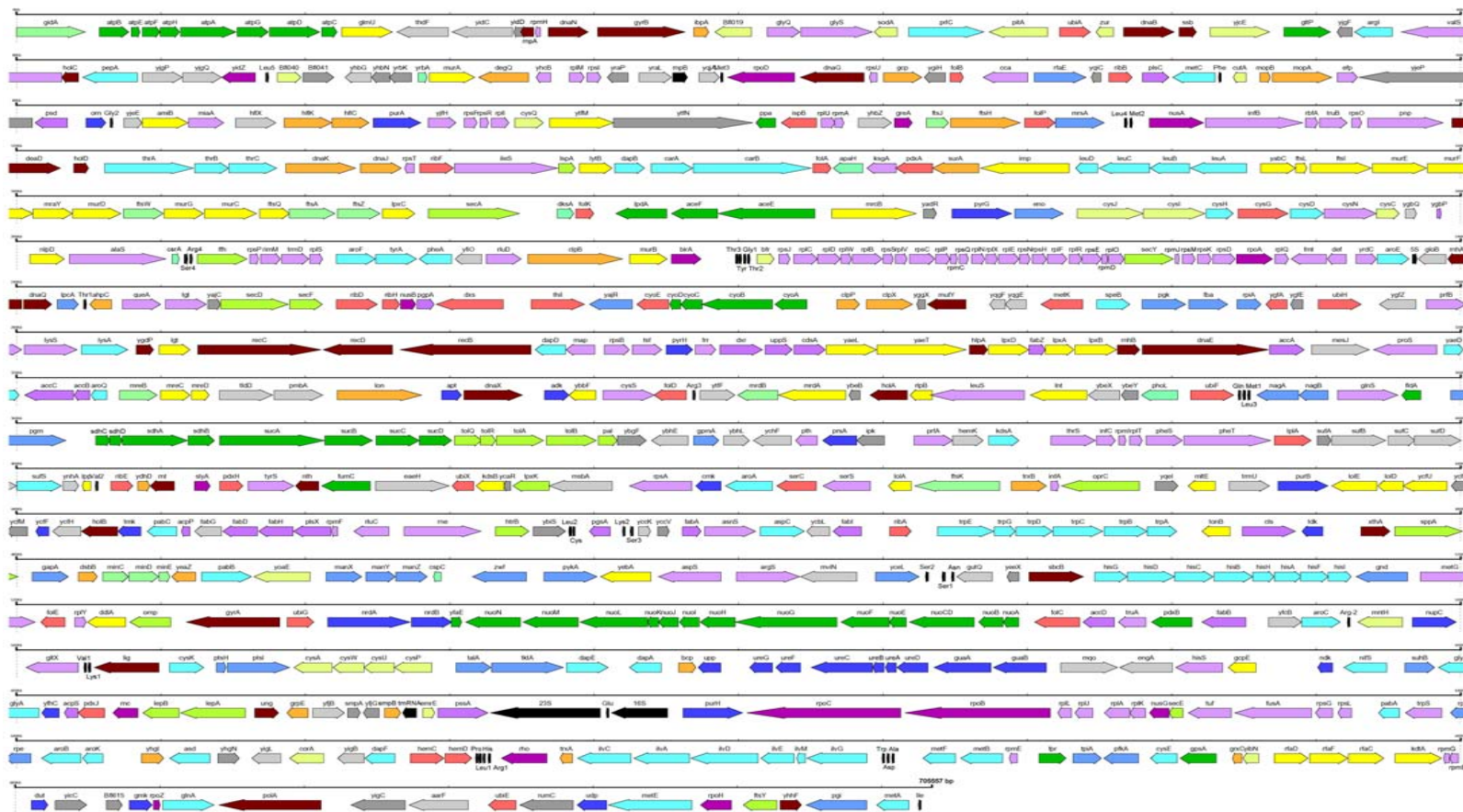


Secuenciación (11.865 lecturas, longitud media = 615 nt)



Ensamblaje (11,238 secuencias, cobertura > 9)







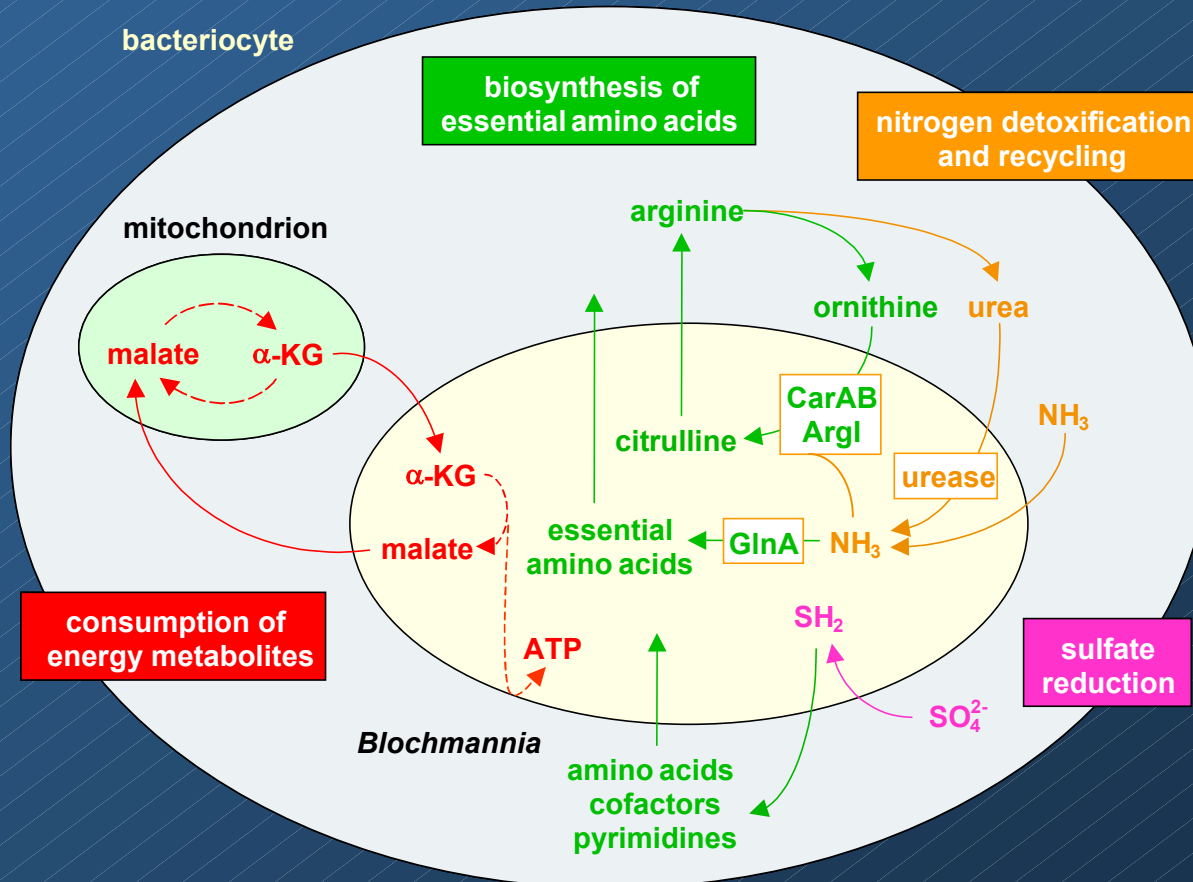
Características generales del genoma

FEATURES	<i>Bl. floridanus</i>	<i>W. glossinidia</i>	<i>Bu. aphidicola</i>		
			BAp	BSg	BBp
Chromosome (bp)	705,557	697,724	640,681	641,454	615,980
Plasmids (total length, bp)	0	1 (5,280)	2 (7,805)	2 (7,967)	1 (2,399)
G+C content (%)	27.38	22	26.2	26.3	25.3
Total gene number	625	661	608	596	545
CDS	583	619	571	559	508
rRNAs	3	6	3	3	3
tRNAs	37	34	32	32	32
Small RNA genes	2	2	2	2	2
Pseudogenes	6	8	12	33	9
Protein-coding regions (%)	83.2	89	86.8	84.5	81.4
Average length ORF (bp)	1,007	988	991	985	992



Análisis funcional

➔ Simbiosis mutualista con base nutricional



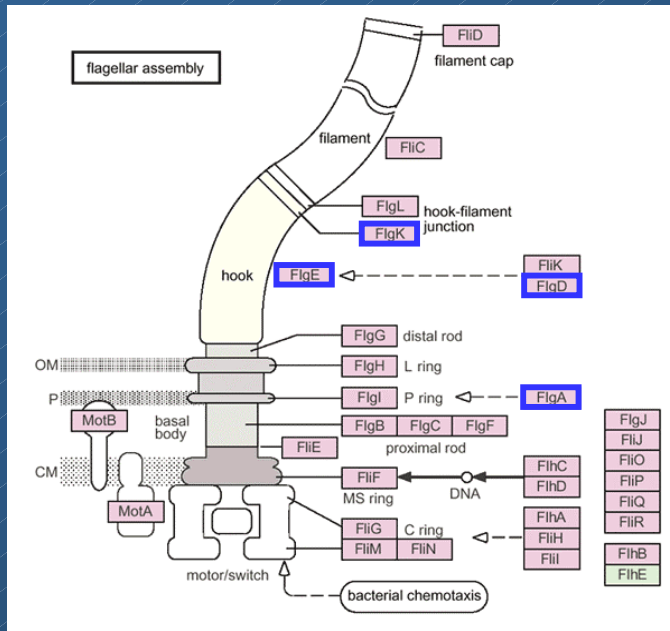


Análisis funcional

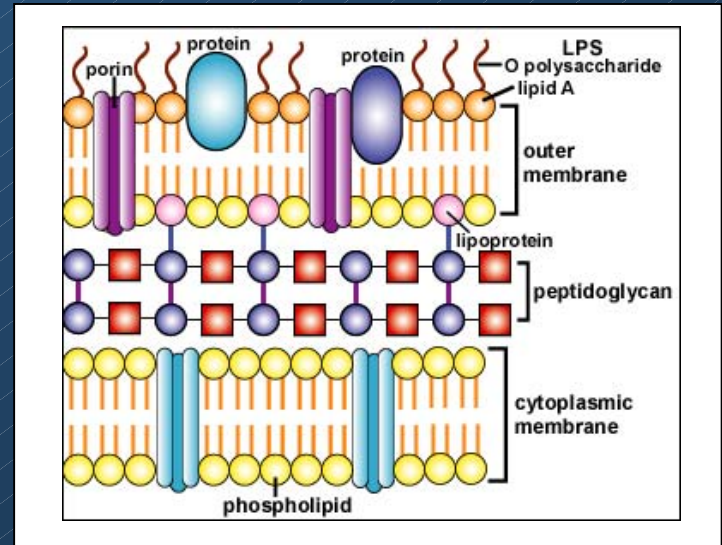
➔ Envuelta celular

- Pérdida de componentes flagelares
- Pared gramnegativa bien estructurada (invasividad, transporte de sustratos)

- sistema de transporte de lipoproteínas
- cluster *tol-pal*



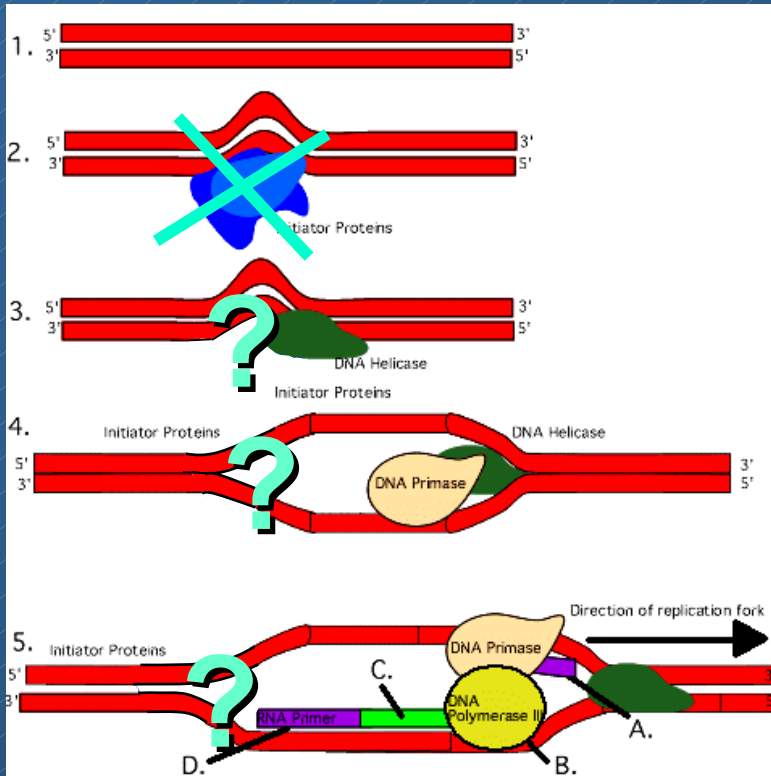
Componentes flagelares de *W. glossinidia*
(Akman et al., 2002)





Análisis funcional

➔ Replicación del DNA



- “Completa” maquinaria de replicación

- DnaB (helicasa)

- DnaG (primasa)

- GyrA/B (girasa)

- DNA polimerasa III

- sin proteína iniciadora (DnaA, RecA, PriA)



Este trabajo ha sido posible gracias a...

The genome sequence of *Blochmannia floridanus*: Comparative analysis of reduced genomes

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Contributed by Bert Hölldobler, June 6, 2002

Bacterial symbioses are widespread among insects, probably being one of the key factors of their evolutionary success. We present the complete genome sequence of *Blochmannia floridanus*, the primary endosymbiont of carpenter ants. Although these ants feed on a complex diet, this symbiont very likely has a nutritional basis. *Blochmannia* is able to supply nitrogen and sulfur compounds to the host while it takes advantage of the host metabolic machinery. Remarkably, these bacteria lack all known genes involved in replication initiation (*oriA*, *polA*, and *recA*). The phylogenetic analysis of a set of conserved protein-coding genes shows that *B. floridanus* is phylogenetically related to *Buchnera aphidicola* and *Megastoma glaberrima*, the other endosymbiotic bacteria whose complete genomes have been sequenced so far. Comparative analysis of the five known genomes from insect endosymbiotic bacteria reveals they share only 313 genes, a number that may be close to the minimum gene set necessary to sustain endosymbiotic life.

Many bacteria live in close association with higher organisms in a symbiotic or parasitic relationship. Whereas much has been learned about pathogenic interactions in the past, little is known about the mechanisms enabling bacteria to have a symbiotic lifestyle. However, symbioses between unicellular and multicellular organisms have contributed significantly to the evolution of life on Earth (1).

Bacterial symbioses are widespread among insects, and it has been estimated that at least 15–20% of all insects live in such symbiotic relationships (2). The early establishment of symbiotic associations among insects and bacteria, ~300 million years ago (3), has probably been one of the key factors for the evolutionary success of insects, because it may have allowed access to novel ecological niches and to new imbalanced food resources, such as plant sap or blood (4). This is the case for the mutualistic and obligate symbiosis of *Buchnera aphidicola* with aphids and of *Megastoma glaberrima* with tsetse flies. These symbiotic bacteria reside in specialized host cells called bacteriocytes, which form symbiotic organs called bacteriomes. The bacterial transmission occurs vertically: the eggs or young embryos are infected by microorganisms derived from the mother.

Most parasitic and symbiotic obligate intracellular bacteria share several genomic features, i.e., bias toward a high A+T content, accelerated sequence evolution (5), and massive genome size reduction with respect to their free-living ancestors (6). This reduction has become so extreme that some *B. aphidicola* strains present the smallest genome sizes (~450 kb) known to date (7), which may represent ~400 protein-coding genes. Comparative analyses of the small size genomes of symbiotic and parasitic bacteria will provide interesting insights into the evolution of resident genomes and the minimum set of genes necessary for intracellular life.

In addition to aphids and tsetse flies, social insects such as ants are particularly interesting for understanding mutualistic relationships, because they have developed numerous interactions

with different species of animals, plants, and microorganisms. Moreover, ants belong to a different insect order than aphids and tsetse flies. The symbiosis of ants of the genus *Crematogaster* with intracellular bacteria (*Blochmannia* spp.), located in the midgut and ovaries of the insects, was the first bacteriocyte endosymbiosis described (8). As in the above-mentioned bacterial endosymbioses of insects, *Blochmannia* spp. generally display concordant evolution with their host species (9). This symbiosis has been described so far only within the members of the subfamily Formicinae, which has an estimated age of ~70 million years, although it is not known whether this symbiosis has been established only in the Formicinae or was an original attribute of ants maintained only in this subfamily (9). Until now, the biological function of this symbiosis remained unknown, because a nutritional basis is not evident at first sight. Although it seems to be a general trend within the genus *Crematogaster* to use honeydew from sap-sucking insects as their main food source, they can feed on a complex diet that may also include dead and live insects, bird excrement, and sweet food waste (10). That adult ants are able to live without their bacterial endosymbionts under laboratory conditions, and that these bacteria seem to degenerate naturally in the course of time, as observed in older queens, suggest that the symbiosis may be of relevance mainly during the early life stages of the ants (11).

Here we present the complete genome sequence of *Blochmannia floridanus*, the primary endosymbiont of the ant *Crematogaster floridanus*, and its comparison with the previously sequenced genomes of four insect endosymbionts and the obligate parasite *Mycobacterium goodii*. The comparative genomics of all known endosymbiotic genomes reveals that they share 313 genes, a number that may be close to the minimum gene set necessary to sustain endosymbiotic life. Furthermore, they share 179 genes with *M. goodii*, which represents a minimum gene set for bacterial cell life.

Materials and Methods

B. floridanus DNA Genomic Purification from Carpenter Ants. *C. floridanus* were maintained in the laboratory at 30°C and fed with honey water and cockroaches.

The bacteriocytes containing the endosymbiont bacteria were purified by an adaptation of the procedure described by Harrison

Data deposition. The sequences reported in this paper have been deposited in the GenBank/EMBL/CCDB database (accession no. BX018551).

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