International Journal of Medical Microbiology 300 (2010) 271–278

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.de/ijmm

Mini Review

Genomics of intracellular symbionts in insects

María José Gosalbes [a,b], Amparo Latorre [a,b], Araceli Lamelas [a,b], Andrés Moya [a,b,*]

[a] Unidad Mixta de Investigación en Genómica y Salud Centro Superior de Investigación en Salud Pública (CSISP), Institut Cavanilles de Biodiversitat i Biologia Evolutiva (Universitat de València), Apartado Postal 22085, 46071 Valencia, Spain
[b] CIBER en Epidemiología y Salud Pública (CIBEResp), Spain

ARTICLE INFO

Article history:
Received 24 October 2009
Received in revised form
3 December 2009
Accepted 20 December 2009

Keywords:
Endosymbiosis
Insects
Genome sequence
Functional genomics

ABSTRACT

Endosymbiotic bacteria play a vital role in the evolution of many insect species. For instance, endosymbionts have evolved metabolically to complement their host's natural diet, thereby enabling them to explore new habitats. In this paper, we will review and give some examples of the nature of the metabolic coupling of different primary and secondary endosymbionts that have evolved in hosts with different nutritional diets (i.e., phloem, xylem, blood, omnivores, and grain). Particular emphasis is given to the evolutionary functional convergence of phylogenetically distant endosymbionts, which are evolving in hosts with similar diets.

Introduction

In recent years, genomics and metagenomics have opened up new avenues of research into symbiotic associations. Metagenomic studies of different habitats, such as sea water, ice cores, marine sponges, or human gut (Venter et al., 2004; Bidle et al., 2007; Schmitt et al., 2007; Booijink et al., 2007) have led to the discovery of the presence of previously unknown and uncultivable bacteria, protozoa, and viruses. In particular, there are increasing numbers of genome sequences for many symbionts, revealing their complete set of genes as well as their functional contribution to their host's metabolism.

Symbiotic associations are particularly well studied in insects. Insects are the most species-rich group of organisms, and it has been estimated that at least 15–20% of all insects live in symbiotic relationships with bacteria (Buchner, 1965; Douglas, 1998). According to the location of the symbiont with respect to the cells, associations can be referred to as ectosymbiotic or endosymbiotic. Among intracellular symbioses, there are differences regarding the extent of dependence between the animal host and the symbiont and the age of the association leading to the distinction between obligate primary endosymbiont (P-endosymbiont) and facultative secondary endosymbiont (S-endosymbiont). S-symbionts are considered facultative since they are not essential to host survival and reproduction even though, in some cases, they confer advantages to their hosts (Oliver et al., 2009). Although they are normally transmitted vertically through host generations, their distribution patterns suggest that sporadic horizontal transmission can occur (Russell et al., 2003; Russell and Moran, 2005). Insects that have established endosymbiotic associations with bacteria are characterized, in general, by feeding upon unbalanced diets, poor in essential nutrients such as amino acids, sterols, or vitamins, which are provided by the symbionts. Other symbionts provide different functions to their host, such as nitrogen recycling and storage or the provision of metabolic factors that are required for survival and fertility (Douglas, 1998; Baumann and Moran, 2000). On the other hand, the bacterium gains a permanent supply of a wide range of metabolites that are provided by the host as well as a safe environment. The first step towards obligate endosymbiosis becoming established is when a free-living bacterium infects the host. Then, both organisms coevolve to adapt to the new situation. On the bacterial side, genomic studies have revealed that the endosymbiont genome gets smaller during this adaptive process, owing to the loss of genes that are rendered unnecessary in the new environment (Gil et al., 2004; Zientz et al., 2004; Moya et al., 2008; Moran et al., 2008; Feldhaar and Gross, 2009).

To date, the following complete genomes of bacterial endosymbionts of insects have been fully sequenced: the endosymbionts of 4 aphid species (*Buchnera* spp.) (Shigenobu et al., 2000; Tamas et al., 2002; van Ham et al., 2003; Pérez-Brocal et al., 2006), 2 S-symbionts of the pea aphid (*Hamiltonella defensa* and *Regiella insecticola*) (Degnan et al., 2009a, 2009b), the endosymbionts of 2 carpenter ant

* Corresponding author at: Unidad Mixta de Investigación en Genómica y Salud Centro Superior de Investigación en Salud Pública (CSISP), Institut Cavanilles de Biodiversitat i Biologia Evolutiva (Universitat de València), Apartado Postal 22085, 46071 Valencia, Spain. Tel.: +34 96 3543480; fax: +34 96 3543670.
E-mail address: andres.moya@uv.es (A. Moya).

1438-4221/$ - see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ijmm.2009.12.001

Table 1
Functional features of intracellular symbionts.

Endosymbiont	Host	Association age	Host nutrition	Genome size (Kb)	Metabolic capacity	Insertion elements	T3SS	Accession number
Buchnera aphidicola Bap (*γ-Proteobacteria*)	*Acyrthosiphon pisum* (aphid)	150 my	Phloem (primary)	652	Amino acids, vitamins	No	Incomplete T3SS	BA000003 AP001070 AP001070
Buchnera aphidicola BSg (*γ-Proteobacteria*)	*Schizaphis graminum* (aphid)	150 my	Phloem (primary)	653	Amino acids, vitamins	No	Incomplete T3SS	AE013218 AF041836 Z21938
Buchnera aphidicola BBp (*γ-Proteobacteria*)	*Baizongia pistaciae* (aphid)	150 my	Phloem (primary)	618	Amino acids, vitamins	No	Incomplete T3SS	AE016826 AF492591
Buchnera aphidicola BCt (*γ-Proteobacteria*)	*Cinara tujafilina* (aphid)	150 my	Phloem (primary)	445	Amino acids, vitamins	No	Incomplete T3SS	–
Buchnera aphidicola BCc (*γ-Proteobacteria*)	*Cinara cedri* (aphid)	150 my	Phloem (primary)	422	Amino acids, Trp incomplete	No	Incomplete T3SS	CP000263 AY438025
[a,b]*Serratia symbiotica* (*γ-Proteobacteria*)	*Cinara cedri* (aphid)	–	Phloem (primary)	In progress	Vitamins, Trp incomplete	No	No	–
Halmitonella defense (*γ-Proteobacteria*)	*Acyrthosiphon pisum* (aphid)	–	Phloem (secondary)	2100	Parasitoid wasp protection	IS elements	2 complete T3SSs	CP001277 CP001278
[a]*Carsonella ruddi* (*γ- Proteobacteria*)	*Pachypsylla venusta* (psyllid)	120 my	Phloem (primary)	160	Symbiotic role lost	No	No	AP009180
Baumannia cicadellinicola (*γ-Proteobacteria*)	*Homalodisca vitripennis* (sharpshooter)	170 my	Xylem (primary)	686	Vitamins, cofactors	No	No	CP000238
[a]*Sulcia muelleri* (*Flavobacteria*)	*Homalodisca vitripennis* (sharpshooter)	> 270 my	Xylem (primary)	246	Amino acids	No	No	CP000770
[a]*Sulcia muelleri* (*Flavobacteria*)	*Diceroprocta semicincta* (singing cicada)	> 270 my	Xylem (primary)	277	Amino acids	No	No	CP001605
[a]*Hodgkinia cicadicola* (*α-Proteobacteria*)	*Diceroprocta semicincta* (singing cicada)	190 my	Xylem (primary)	144	Amino acids	No	No	NC012960
Wigglesworthia glossinidia (*γ-Proteobacteria*)	*Glossina brevipalpis* (tsetse fly)	40–80 my	Blood (primary)	698	Vitamins, cofactors	No	complete T3SS	BA000021 AB063523
Sodalis glossinidius (*γ-Proteobacteria*)	*Glossina morsitans* (tsetse fly)	–	Blood (secondary)	4171	Immunity	IS elements	3 complete T3SSs	AP008232
[a]*Blochmannia pennsylvanicus* (*γ-Proteobacteria*)	*Camponotus pennsylvanicus* (carpenter ant)	50 my	Omnivore (primary)	792	Nitrogen metabolism	No	No	CP000016
[a]*Blochmannia floridanus* (*γ-Proteobacteria*)	*Camponotus floridanus* (carpenter ant)	50 my	Omnivore (primary)	706	Nitrogen metabolism	No	No	BX248583
Blattabacterium (*Flavobacteria*)	*Blattella germanica* (cockroach)	150 my	Omnivore (primary)	637	Nitrogen metabolism	No	No	CP001487
Blattabacterium (*Flavobacteria*)	*Periplaneta americana* (cockroach)	150 my	Omnivore (primary)	637	Nitrogen metabolism	No	No	CP001429
SOPE (*γ-Proteobacteria*)	*Sitophilus oryzae* (weevil)	Less than 25 my	Grain (primary)	3000[c]	Amino acids, vitamins	IS elements	Complete T3SS	–

my, Millions of years.

[a] Candidatus.
[b] Secondary endosymbiont in other aphids.
[c] Size estimated by pulse-field gel.

species (*Blochmannia* spp.) (Gil et al., 2003; Degnan et al., 2005), one symbiont of psyllids (“*Candidatus* Carsonella ruddii”) (Nakabachi et al., 2006), the P-endosymbiont (*Wigglesworthia glossinidia*) and the S-endosymbiont (*Sodalis glossidinus*) of tsetse flies (Akman et al., 2002; Toh et al., 2006), 2 different endosymbionts coresiding in a xylem-feeding sharpshooter and singing cicadas (“*Candidatus* Sulcia muelleri”, *Baumannia cicadellinicola*, and “*Candidatus* Hodgkinia cicadicola”) (McCutcheon and Moran, 2007; McCutcheon et al., 2009a, 2009b), and a *Blattabacterium* strain from the cockroach *Blattella germanica* (López-Sánchez et al., 2009) as well as from *Periplaneta americana* (Sabree et al., 2009). Furthermore, 5 genomes of *Wolbachia*, a widespread intracellular α-proteobacterium, have being sequenced (Wu et al., 2004; Foster et al., 2005; Klasson et al., 2008, 2009; Salzberg et al., 2009). In this review, we will focus on the functional interdependencies between partners from the data provided by genomic analyses (Table 1).

Does insect diet lead the evolutionary pathway towards minimal metabolism?

Genomic and metagenomic analyses have corroborated the nutritional role of such associations, as in each case the bacteria provide the nutrients lacking in the respective host diets. The host has, so to speak, domesticated the endosymbiont, so it devotes part of its gene repertoire to processes that are more beneficial to its host than to itself.

Phloem diet

Several insects are plant phloem-feeding insects, a diet that is rich in sugars but poor in nitrogenous compounds such as amino acids, vitamins, or cofactors. This is the case of aphids, psyllids, whiteflies, and mealybugs. They have established endosymbiotic associations with different bacteria (*Buchnera aphidicola*, “*Candidatus* Carsonella ruddii”, “*Candidatus* Portiera aleyrodidarum” and “*Candidatus* Temblaya princeps”, respectively) (Thao et al., 2000; Munson et al., 1991; Tremblay, 1989; Clark et al., 1992), which provide the nutrients lacking in each case.

Buchnera aphidicola and its aphid hosts are the best-studied insect–symbiont associations. Nowadays, 4 complete genomes of *Buchnera aphidicola* from 4 different aphids (*Acyrthosiphon pisum: B. aphidicola* BAp, *Schizaphis graminum: B. aphidicola* BSg, *Baizongia pistacea: B. aphidicola* BBp, and *Cinara cedri: B. aphidicola* BCc) have been fully sequenced (Shigenobu et al., 2000; Tamas et al., 2002; van Ham et al., 2003; Pérez-Brocal et al., 2006; Moran et al., 2009). Furthermore, sequencing of the genome of *B. aphidicola* BCt from *Cinara tujafilina* is currently underway in our laboratory. As a consequence of its association with the aphid, the bacterial genome has undergone a prominent size reduction. *Buchnera* genome sequences revealed that some of the genes lost were those required for the synthesis of the nonessential amino acids that the aphid can make themselves. Aphids require 10 essential amino acids that are lacking in their phloem-sap diet and must be provided by their endosymbionts. In fact, nearly 10% of *B. aphidicola* coding capacity is devoted to amino acid biosynthesis. In 4 out of the 5 of the aphid lineages studied, the structural genes for leucine biosynthesis, and 2 regulatory genes (*trpEG*) of the tryptophan pathway are located in plasmids, while the rest of the genes of this pathway remain in the chromosome (Lai et al., 1994; Van Ham et al., 1999; Gil et al., 2006; Pérez-Brocal et al., 2006). *B. aphidicola* from *C. cedri* has retained the biosynthetic capacity for all essential amino acids except tryptophan, whose pathway is incomplete (Gosalbes et al., 2008). The case of tryptophan in *C. cedri* will be discussed below.

Another characteristic feature of *Buchnera* genomes is the presence of a simplified flagellar apparatus, homologous to the type III secretion system (T3SS). Since *Buchnera* species are not motile, it has been proposed that these structures might be involved in invading bacteriocytes to ensure transmission to host offspring, and also a possible protein export function has been described (Shigenobu et al., 2000; Pérez-Brocal et al., 2006: Toft and Fares, 2008). Comparison of the available genome sequences reveals deletions and inactivation of ancestral genes in each lineage.

B. aphidicola BSg possesses degenerate copies of genes underlying incorporation of inorganic sulphur, because the incorporation of this compound is unnecessary since the aphid’s grass diet provides the sulphur-containing organic compounds required to produce methionine and cysteine (Tamas et al., 2002; Moran, 2007). This loss implies that *S. graminum* is destined to remain on grasses or other hosts with sufficient sources of fixed sulphur (Tamas et al., 2002). Similar losses have occurred in *B. aphidicola* BBp, which lacks the pathway for arginine and pantothenate biosynthesis but retains genes underlying biotin biosynthesis, which have been lost in genomes of *B. aphidicola* from *S. graminum*, *A. pisum*, *C. tujafilina*, and *C. cedri* (van Ham et al., 2003; Pérez-Brocal et al., 2006; Lamelas et al., unpublished). Hence, some of the observed gene losses are expected to affect host ecology.

In addition to *B. aphidicola*, some aphid populations harbour facultatively symbiontic intracellular bacteria. In the aphid *C. cedri,* the S-endosymbiont “*Candidatus* Serratia symbiotica” (hereafter, *S. symbiotic*a) is reported to have become an obligated symbiont (Lamelas et al., 2008). Indeed, there are recent reports of a close endosymbiotic consortium that involves *B. aphidicola* and *S. symbiotica* in *C. cedri* (Gosalbes et al., 2008) (Fig. 1). Applying a metagenomic approach, we discovered a plasmid in *B. aphidicola* from *C. cedri* containing the *trpEG* genes, coding for anthranilate synthase, the first enzyme of the tryptophan biosynthesis pathway. This plasmid showed homology with all the tryptophan plasmids described previously for several *B. aphidicola* strains (Lai et al., 1994; Van Ham et al., 1999; Gil et al., 2006). The remaining genes for the pathway (*trpDCBA*) are located on the chromosome of *S. symbiotica*. These data show that both endosymbionts, *B. aphidicola* and *S. symbiotica*, are involved in the tryptophan biosynthesis that supplies this essential amino acid to both their host and themselves. Therefore, the obligate biochemical interdependence between 2 endosymbionts can represent an evolutionary seal of the bacterial metabolic complementation, and the establishment of a stable consortium would be the expected evolutionary outcome.

Another facultative symbiont, *H. defensa,* occurs in aphids and others sap-feeding insects such as psyllids or whiteflies. This S-endosymbiont protects its host by killing the parasitoid wasp larvae and allowing the insect to survive and reproduce. In the pea aphid (*A. pisum*), the degree of protection varies among *Hamiltonella* strains and depends on toxins that are produced by the bacteria and by a temperate lambda-like bacteriophage, which infects *H. defensa*. The complete genome sequence (2.1 Mb) of *H. defensa* from *A. pisum* has a complete inventory of pathogenicity factors such as 2 T3SS or diverse putative toxins (Moran et al., 2005; Degnan et al., 2009a). The genome also presents mobile DNA and insertion-sequence elements, indicating a dynamic nature and horizontal gene transfer. Related to the metabolism, this endosymbiont is host-dependent, synthesizing only 2 of the essential amino acids and most vitamins except thiamine and pantothenate. The diet requirements of both *H. defensa* and the aphid must rely on another endosymbiont, mainly *Buchnera*.

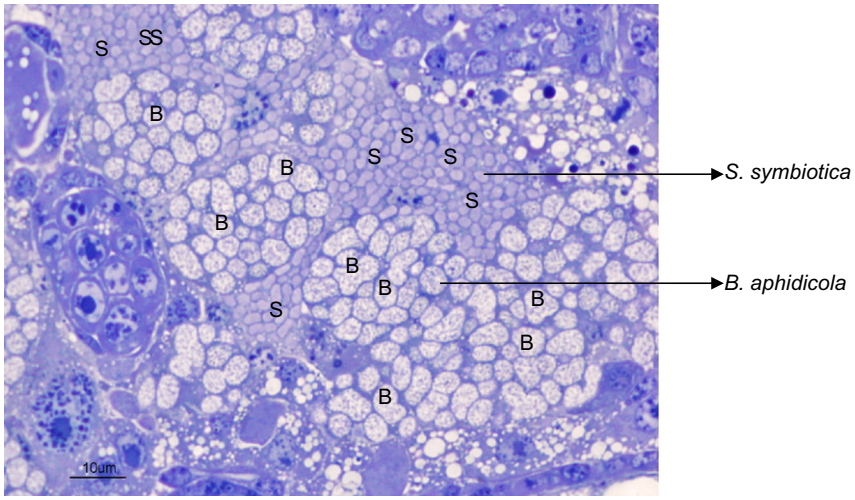

Fig. 1. Ultrathin section from *Cinara cedri* showing the bacteriocytes of the 2 symbionts that form the functional consortium. B, *Buchnera aphidicola*; S, *Serratia symbiotica*.

Psyllids are the other phloem sap-feeding insects, and the genome sequence of their primary endosymbiont, "*Candidatus* Carsonella ruddii" (hereafter *C. ruddii*), has been obtained (Thao et al., 2000; Nakabachi et al., 2006). The genome sequencing of *C. ruddii* from the psyllid *Pachrypsylla venusta* revealed that it possess a 160-kb genome with only 182 predicted open reading frames, with many genes been lost (Nakabachi et al., 2006). In fact, about half of the biosynthesis pathways for essential amino acids are either partially or entirely missing, indicating a severe impairment of its symbiotic function. Since no secondary endosymbionts appear to be associated with these psyllids, it is difficult to understand how the hosts can thrive on their diet without additional symbiotic help. Furthermore, the ability of the cell to perform the most essential life-related functions is heavily impaired by the lack of genes involved in DNA replication, transcription, and translation. Therefore, it has yet to be clarified whether *C. ruddii* is on its way to becoming an organelle, or if it is nearing extinction and replacement by an unidentified symbiont (Tamames et al., 2007).

Xylem diet

Xylem is the component of the plant vascular system that is used to transport water and salts from the roots to the rest of the plant. Xylem sap is low in nutrients, containing mostly inorganic compounds and minerals, as well as small amounts of non-essential amino acids (glutamate, aspartate), sugars (primarily glucose), and organic acids (primarily malate) (Redak et al., 2004). Among xylem-feeders, sharpshooters are a prominent group that is endowed with a particular endosymbiotic community, since they possess 2 endosymbionts: the γ-proteobacteria *B. cicadellinicola* and "*Candidatus* Sulcia muelleri" (hereafter *S. muelleri*) from the *Bacteroidetes* phylum. Recently, the 2 complete genome sequences of the endosymbionts have been described from the glassy-winged sharpshooter *Homalodisca vitripennis* (formerly *H. coagulata*). The genome of *B. cicadellinicola* is 686 kb, and 605 protein-coding genes have been identified while *S. muelleri* with a smaller genome (245 kb) shows 228 open reading frames (Wu et al., 2006; McCutcheon and Moran, 2007). *Baumannia* is considered to be like a vitamin and cofactor machine in this system. A large fraction of its genome (83 genes) is involved in pathways for the synthesis of vitamins (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, and folic acid), cofactors, and prosthetic groups. However, these γ-proteobacteria present a very limited set of essential amino acid biosynthesis pathways. The few capabilities that are present include histidine biosynthesis and synthesis of methionine if external homoserine is provided. This loss implies that both the host and *Baumannia* must obtain the amino acids from another external source. In this respect, *S. muelleri* plays an important role, producing 8 (lysine, valine, threonine, leucine, isoleucine, phenylalanine, arginine, tryptophan) of the 10 essential amino acids, despite having a small genome. The lack of vitamin and cofactor synthesis pathways in *Sulcia* indicated that both endosymbionts play complementary and not overlapping roles in the symbiotic system. Not only do they appear to provide different resources for the host, but each does not present the biosynthesis pathways corresponding to the resources synthesized by the other. A similar metabolic consortium has been observed, as described above, in phloem-feeding *C. cedri* with *B. aphidicola* and *S. symbiotica* as endosymbionts. The complementarity between the host and each symbiont extends to mutual dependence between the symbionts that appear to depend on each other for the required compounds and for intermediaries in the metabolic processes.

Recently, a similar metabolic system has been described in singing cicadas, related to the glassy-winged sharpshooter (McCutcheon et al., 2009a). They feed exclusively on xylem sap from plant roots during their long underground juvenile stage, and they also harbour 2 endosymbionts: *S. muelleri* (272 kb) and an α-proteobacterium "*Candidatus* Hodgkinia cicadicola" (hereafter *H. cicadicola*) with the smallest genome described (144 kb). The *H. cicadicola* genome displays some other atypical features such as a high GC content (58.4%) and a recoding of UGA Stop-Trp (McCutcheon et al., 2009b). The *Sulcia* genome has nearly the same gene content and gene order as *Sulcia* from sharpshooter. In cicadas, this microorganism produces 8 of the essential amino acids while the remaining 2 are provided by *Hodgkinia.* Although they belong to different bacterial groups, *Baumannia* (*γ-Proteobacteria*) and *Hodgkinia* (*α-Proteobacteria*) play the same functional role for amino acid synthesis, except for the methionine biosynthetic pathway. *Hodgkinia* has lost 2 genes (*metA* and *metB*) essential to cystathione synthesis, an intermediary of the methionine pathway, as cicada feed on plant root exudate rich in this compound. Another striking feature is the use of cobalamin-dependent methionine synthase (MetH) by *Hodgkinia* instead of MetE (cobalamin-independent methionine synthase) for the last step in methionine biosynthesis like the other insect symbionts. The preference for MetH implies the

presence of cobalamin biosynthesis genes that have never been found in other symbiont genomes before. Unlike *Baumannia, Hodgkinia*, with a drastically reduced genome, has lost all vitamin and cofactor biosynthetic abilities, implying that the cicada and its symbionts must have access to external sources of these compounds, possibly from plant root xylem since cicadas spend most of their lives underground. On the other hand, the translational apparatus is incomplete in *Sulcia* and *Hodgkinia*, and it is still unknown whether this process occurs in these microorganisms as it does in *C. ruddii*.

Blood diet

Tsetse flies (*Glossina* spp.) are the vector for the trypanosome parasite, agents of sleeping sickness in humans and nagana in animals. These insects feed exclusively on vertebrate blood, which is vitamin deficient, mainly in B-complex vitamins. Tsetse flies harbour 2 symbiotic bacteria in gut tissue that are maternally transmitted to intrauterine larvae via milk secretions of the mother. *W. glossinidia* (*γ-Proteobacteria*) is the obligate primary endosymbiont and *S. glossinidius* (*γ-Proteobacteria*) is the commensal secondary symbiont (Aksoy, 1995). Some populations of tsetse flies also harbour a third organism that belongs to the genus *Wolbachia* (*α-Proteobacteria*) (Cheng et al., 2000). The complete genome sequence (698 kb) of *W. glossinidia* shows that the endosymbiont has retained 62 genes (10% of coding sequences) devoted to the biosynthesis of cofactors, prosthetic groups, and carriers, including B vitamins (Akman et al., 2002). As in the previous cases, this fact coupled with data from dietary supplementation experiments of antibiotic-fed, symbiont-free flies, show that the ability to reproduce was partially restored (Nogge, 1976). The small genome of *Wigglesworthia* presents the machinery for the synthesis of a complete flagellar apparatus. However, neither flagellum nor motility has been observed in this endosymbiont. Since the genome does not seem to encode a secretion system to penetrate the larval cells, the flagellar structure may work like a T3SS, as described in the well-established P-endosymbiont *Buchnera* (Pérez-Brocal et al., 2006; Aksoy and Rio, 2005).

S. glossinidius is the S-endosymbiont residing intra- and extracellularly in tsetse flies, principally in the midgut tissue, and it is the only insect symbiont to have been cultured (Matthew et al., 2005). The *Sodalis* genome (4 Mb) has reduced in size compared to its free-living relatives, but it is significantly larger than those of P-symbionts. In addition, it has 3 extrachromosomal plasmids and in some strains a bacteriophage-like element (Darby et al., 2005). The chromosome also contains an unusually high number of pseudogenes, suggesting a degradation of the genes whose function is no longer needed by this organism (Akman et al., 2002; Toh et al., 2006). All these features indicate its recent symbiotic affiliation. This symbiont has apparently retained many of the abilities of free-living bacteria, including vitamin and cofactor biosynthesis pathways. Thus, *Sodalis* benefits its tsetse host via the synthesis of these compounds, which are deficient in a blood diet, as well as *W. glossinidia*, the primary endosymbiont. Meanwhile, these bacteria have lost genes for carbon compound catabolism, central intermediary metabolism and, fatty acid phospholipid metabolism. In general, *Sodalis* seems to have remained synthetic rather than degradative, showing this bacterium has adapted to the energy-rich environment and nutritional ecology of the host insect, i.e. blood. The *Sodalis* chromosome encodes 3 putative T3SS and, as occurring in other symbionts, they have been related to progeny transmission and the establishment of symbiosis (Toh et al., 2006). In experiments where *Sodalis* were selectively eliminated from tsetse, the trypanosome infection rate was significantly reduced. Thus, *Sodalis* plays a role in the efficacy of the host's immune system. Since *Sodalis* can be cultivated in vitro, a new genetic transformation system, paratransgenesis, has recently been developed, by which foreign products are inserted and expressed in *Sodalis* and, in turn, expressed in the tsetse flies; furthermore, they are passed on to multiple generations. This technique can be used to diminish the vectorial capacity of flies by manipulating the relationship between host (tsetse fly), parasite (trypanosome), and endosymbiont (*Sodalis*) (Dale and Welburn, 2001; Aksoy and Rio, 2005; Aksoy et al., 2008).

Another interesting case of an insect with blood diet is the mosquito *Aedes aegypti*, the primary vector of dengue virus. *Wolbachia* species are obligatory endosymbionts of a wide range of invertebrates, including mosquitoes, fruit flies, and filarial nematodes. Recently, *A. aegypti* has been artificially infected with *Wolbachia pipientis* that reduces the lifespan of insects (McMeniman et al., 2009). Turley et al. (2009) have shown that the old *Wolbachia*-infected mosquitoes present also a limited blood-feeding capability. Hence, *Wolbachia* infection could be considered as a biocontrol strategy given that only old mosquitoes transmit dengue.

Omnivorous diet

Ants, social insects, have motivated great interest due to their evolutionary success in terms of species richness and their extraordinary abundance in tropical regions. These insects have also managed to spread into extreme habitats, such as arctic regions or deserts. In the species-rich genus *Camponotus*, carpenter ants, the intracellular bacterium *Blochmannia* has been described, located in bacteriocytes intercalated between midgut cells and in ovaries of females (Blochmann, 1892). The diet of *Camponotus* ranges from nutrient-deficient plant secretions or Homoptera exudate to complex sources like dead insects, bird excrement, or sweet food waste. Thus, given the ants' omnivorous diet, the nutritional role of *Blochmannia* was not clear. However, the genome sequences of "*Candidatus* Blochmannia floridanus" and "*Candidatus* Blochmannia pennsylvanicus" (hereafter *Blochmannia floridanus* and *Blochmannia pennsylvanicus*, respectively) showed that these endosymbionts contribute to nitrogen, sulphur, and lipid metabolism of the host (Gil et al., 2003; Degnan et al., 2005). A prominent feature of ant endosymbionts is the presence of a complete urease gene cluster (Feldhaar et al., 2007). This enzyme hydrolyzes urea to CO_2 and ammonia. This latter compound is a potent cell poison that is recycled by glutamine synthetase into amino acid before toxic concentrations are accumulated. *Blochmannia* has retained the biosynthetic pathways for essential amino acids (except arginine), while those for several non-essential amino acids have been lost. With respect to arginine amino acid, the endosymbiont presents the enzymes that catalyze citrulline synthesis from ornithine. Thus the endosymbiont helps the ants to recycle nitrogen compounds in developmental phases, such as metamorphosis when high anabolic activities support little or no uptake of substrates. When required, the stored nitrogen may be mobilized by the action of host cell-derived arginases and *Blochmannia* urease. In some pathogenic microorganisms, ureases have been identified as virulence factors, whereas in this symbiotic association, it has become beneficial. Therefore, *Blochmannia* confers a significant fitness advantage to its host by enhancing its competitiveness when compared with other ant species lacking this endosymbiont. Unlike other endosymbionts, *Blochmannia* has lost the flagellar apparatus that has been described as both a transport system and invasion mechanism of bacteriocytes, ovaries, or embryos.

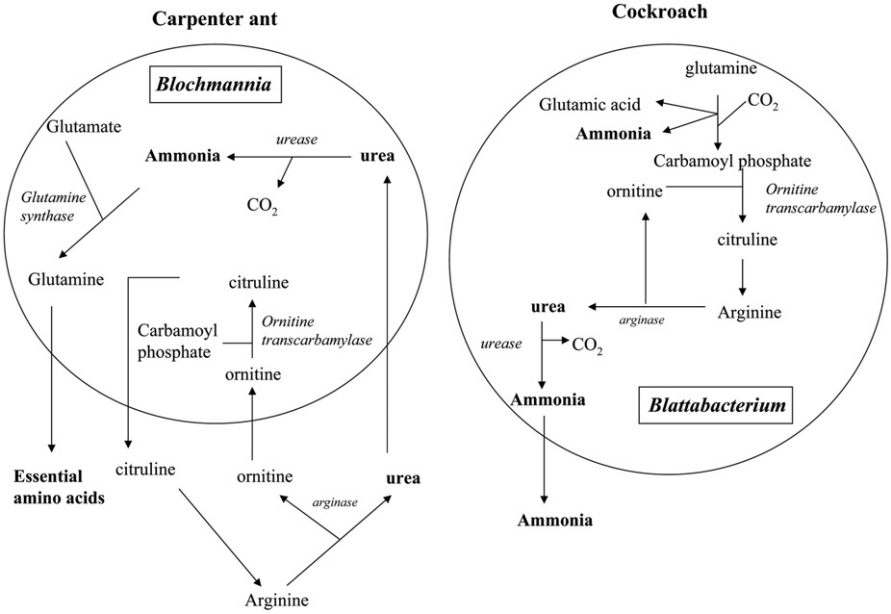

Fig. 2. Comparison of nitrogen recycling metabolism deduced from the genome sequences of *Blochmannia floridanus* and *Blattabacterium* from *Camponotus floridanus* and *Blattella germanica*, respectively.

Cockroaches, like ants, have an omnivorous diet. They harbour an endosymbiont, *Blattabacterium*, in bacteriocytes located in their abdominal fat body. *Blattabacteria* belong to the class *Flavobacteria* in the phylum *Bacteroidetes*. Studies into genome size have revealed the genome sizes of the endosymbionts of 3 cockroach species, *Periplaneta americana, Blatta orientalis*, and *Blattella germanica*, to be approximately 650 kb (López-Sánchez et al., 2008). Recently, the genome sequences of *Blattabacterium* strain Bge and *Blattabacterium* sp. BPLAN, the primary endosymbionts of *B. germanica* and *P. americana*, respectively, have been determined and shown to have both a genome size of 637 kb (López-Sánchez et al., 2009; Sabree et al., 2009). *Blattabacterium* strain Bge genome presents a striking trait, a complete urea cycle. This feature has been described in only one member of the *Bacteroidetes* phylum, a cellulolytic soil bacterium (Xie et al., 2007). Regardless of the phylogenetic distance, *Blattabacterium* shares more genes with *Blochmannia*, a γ-proteobacterial endosymbiont of carpenter ants, than with other endosymbionts. These data revealed a functional convergence that depends on the host's diet. However, *Blattabacterium* lacks the genes encoding glutamine synthetase, an essential enzyme in nitrogen conversion during amino acid anabolism. Thus, the function of urease is not the same as in carpenter ant endosymbionts. *Blochmannia* strains produce ammonia from dietary urea and then use it as a source of nitrogen, whereas the *Blattabacterium* strain codes for the complete urea cycle that, in combination with urease, produces ammonia as an end product (Fig. 2). In addition, the cockroach endosymbionts, *Blattabacterium* strains Bge and BPLAN, have genes encoding essential amino acids as well as cofactors and vitamins and they are involved in sulphate assimilation into sulphur amino acids.

Grain diet

Grain is also an unbalanced diet, very rich in starch and carbohydrates but deficient in other nutrients. Grain weevils (*Sitophilus* species) harbour a primary endosymbiont that lives in bacteriocytes located surrounding the fore-midgut junction of the insect and in the apex of female ovaries. The best studied primary endosymbionts are SOPE and SZPE from *Sitophilus oryzae* and *S. zeamais*, respectively. SOPE, *γ-Proteobacteria*, maintains an obligate mutualistic endosymbiosis with the host and has not been cultured outside the weevil. Experiments based on heat treatments eliminated SOPE from the host, thus enabling its role within the association to be studied. These bacteria provide amino acids such as phenylalanine and proline as well as vitamins (Heddi et al., 1999). Comparative genomics using microarray analysis showed that in this endosymbiont the greatest gene losses have occurred in those involved in cellular processes (Rio et al., 2003). The SOPE genome has been estimated by pulse-field gel electrophoresis at about 3 Mb, without observing drastic genome size reduction (Charles et al., 1997). Phylogenetic studies indicate that SOPE is closely related to *S. glossinidius* (Heddi et al., 1998). Partial sequencing of the SOPE and SZPE genomes shows a large number of insertion sequences, indicating that they have recently acquired an obligate intracellular way of life (Gil et al., 2008; Plague et al., 2007). Furthermore, it has been proposed that the grain weevil symbionts replaced a more ancient symbiont, which is still retained in other weevil groups (Lefèvre et al., 2004). The presence of closely related T3SS-encoding genes has been found in *Sodalis* and SZPE, and their expression is coincident with the timing of bacteriome infection in the developing weevil. Thus, T3SS was acquired by a common ancestor of SPZE and *Sodalis* and was retained as an essential element in the establishment and maintenance of infection and evolved in symbiotic relationships with different hosts that have distinct ecological niches and different specialized diets.

Perspectives and conclusions

Several genome sequencing projects of intracellular symbionts are underway. Recently, the P-endosymbiont of primate lice (*Pediculus humanus*) with a keratin diet has been characterized, and the genome is currently being sequenced (Allen et al., 2007; Nováková et al., 2009). "*Candidatus* Midichloria mitochondrii" is an intracellular symbiont of the tick *Ixodes ricinus*, for which a sequencing project is being carried out (Sassera et al., 2006, 2008). Mealybugs are plant sap-sucking insects which have a

prokaryote–prokaryote endosymbiosis, found nowhere else in nature. *Tremblaya princeps*, a β-proteobacterium, harbours within its cell other Gram-negative bacteria (secondary endosymbiont) belonging to the *γ-Proteobacteria*. Metagenomic analyses of this association will lead to an understanding of the nutritional, physiological, and ecological interactions between their members (Baumann et al., 2002; Kono et al., 2008). The whitefly *Bemisia tabaci* harbours *Portiera aleyrodidarum*, an obligatory symbiotic bacterium, as well as several secondary symbionts including *Rickettsia*, *Hamiltonella*, *Wolbachia*, *Arsenophonus*, *Cardinium*, and *Fritschea*, the function of which has yet to be studied in depth. *B. tabaci* is a species complex composed of numerous biotypes, which may differ from each other both genetically and biologically, and where the different biotypes carry different S-endosymbionts (Baumann et al., 2004; Chiel et al., 2007). In summary, genomics and metagenomics constitute powerful tools that can be used in the analyses of complex symbiotic consortia, in order to understand their role in the dynamics and evolution of insect populations.

Acknowledgements

Financial support was provided by grants BFU2006/06003/BMC and BFU2009-12895-C02-01/BMC (Ministerio de Ciencia e Innovación, Spain) to A. Latorre and the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement number 212894 and Prometeo/2009/092 (Conselleria d'Educació, Generalitat Valenciana, Spain) to A. Moya. A. Lamelas is funded by a predoctoral fellowship from the Generalitat Valenciana (Spain).

References

Akman, L., Yamashita, A., Watanabe, H., Oshima, K., Shiba, T., Hattori, M., Aksoy, S., 2002. Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. Nat. Genet. 32, 402–407.

Aksoy, S., 1995. *Wigglesworthia* gen. nov. and *Wigglesworthia glossinidia* sp. nov., taxa consisting of the mycetocyte-associated, primary endosymbionts of tsetse flies. Int. J. Syst. Bacteriol. 45, 848–851.

Aksoy, S., Rio, R.V.M.T., 2005. Interactions among multiple genomes: Tsetse, its symbionts and trypanosomes. Insect Biochem. Mol. Biol. 35, 691–698.

Aksoy, S., Weiss, B., Attardo, G., 2008. Paratransgenesis applied for control of tsetse transmitted sleeping sickness. Adv. Exp. Med. Biol. 627, 35–48.

Allen, J.M., Reed, D.L., Perotti, M.A., Braig, H.R., 2007. Evolutionary relationships of "*Candidatus* Riesia spp.," endosymbiotic *Enterobacteriaceae* living within hematophagous primate lice. Appl. Environ. Microbiol. 73, 1659–1664.

Baumann, L., Thao, M.L., Hess, J.M., Johnson, M.W., Baumann, P., 2002. The genetic properties of the primary endosymbionts of mealybugs differ from those of other endosymbionts of plant sap-sucking insects. Appl. Environ. Microbiol. 68, 3198–3205.

Baumann, L., Thao, M.L., Funk, C.J., Falk, B.W., Ng, J.C., Baumann, P., 2004. Sequence analysis of DNA fragments from the genome of the primary endosymbiont of the whitefly *Bemisia tabaci*. Curr. Microbiol. 48, 77–81.

Baumann, P., Moran, N.A., 2000. Bacteriocyte-associated endosymbionts of insects. In: Dworkin, M. (Ed.), The Prokaryotes. Springer Verlag, New York, pp. 1–67.

Bidle, K.D., Lee, S., Marchant, D.R., Falkowski, P.G., 2007. Fossil genes and microbes in the oldest ice on Earth. Proc. Natl. Acad. Sci. USA 33, 13455–13460.

Blochmann, F., 1892. Über das Vorkommen von bakterienähnlichen Gebilden in den Geweben und Eiern verschiedener Insekten. Zentralbl. Bakteriol. 11, 234–240 in German.

Booijink, C., Zoetendal, E.G., Kleerebezem, M., de Vos, W.M., 2007. Microbial communities in the human small intestine: coupling diversity to metagenomics. Future Microbiol. 3, 285–295.

Buchner, P., 1965. Endosymbiosis of Animals with Plant Microorganisms. Interscience Publishers, New York.

Charles, H., Condemine, G., Nardon, C., Nardon, P., 1997. Genome size characterization of the endocellular symbiotic bacteria of the weevil *Sitophilus oryzae*, using pulse field gel electrophoresis. Insect Biochem. Mol. Biol. 27, 345–350.

Cheng, Q., Ruel, T.D., Zhou, W., Moloo, S.K., Majiwa, P., O'Neill, S.L., Aksoy., S., 2000. Tissue distribution and prevalence of *Wolbachia* infections in tsetse flies, *Glossina* spp. Med. Vet. Entomol. 14, 44–50.

Chiel, E., Gottlieb, Y., Zchori-Fein, E., Mozes-Daube, N., Katzir, N., Inbar, M., Ghanim, M., 2007. Biotype-dependent secondary symbiont communities in sympatric populations of *Bemisia tabaci*. Bull. Entomol. Res. 97, 407–420.

Clark, M.A., Baumann, L., Munson, M.A., Baumann, P., Campbell, B.C., Duffus, J.E., Osborne, L.S., Moran, N.A., 1992. The eubacterial endosymbionts of whiteflies (Homoptera: Aleyrodoidea) constitute a lineage separated from the endosymbionts of aphids and mealybugs. Curr. Microbiol. 25, 119–123.

Dale, C., Welburn, S.C., 2001. The endosymbionts of tsetse flies: manipulating host-parasite interactions. Int. J. Parasitol. 31, 628–631.

Darby, A.C., Lagnel, J., Matthew, C.Z., Bourtzis, K., Maudlin, I., Welburn, S.C., 2005. Extrachromosomal DNA of the symbiont *Sodalis glossinidius*. J. Bacteriol. 187, 5003–5007.

Degnan, H., Lazarus, A.B., Wernegreen, J.J., 2005. Genome sequence of *Blochmannia pennsylvanicus* indicates parallel evolutionary trends among bacterial mutualists of insects. Genome Res. 15, 1023–1033.

Degnan, P.H., Yu, Y., Sisneros, N., Wing, R., Moran, N.A., 2009a. *Hamiltonella defensa*, genome evolution of protective bacterial endosymbiont from pathogenic ancestors. Proc. Natl. Acad. Sci. USA 106, 9063–9068.

Degnan, P.H., Leonardo, T.E., Cass, B.N., Hurwitz, B., Stern, D., Gibbs, R.A., Richards, S., Moran, N.A., 2009b. Dynamics of genome evolution in facultative symbionts of aphids.. Environ. Microbiol. 10.1111/j.1462-2920.2009.02085.x.

Douglas, A.E., 1998. Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. Annu. Rev. Entomol. 43, 17–37.

Feldhaar, H., Gross, R., 2009. Insects as hosts for mutualistic bacteria. Int. J. Med. Microbiol. 299, 1–8.

Feldhaar, H., Straka, J., Krischke, M., Berthold, K., Stoll, S., Mueller, M.J., Gross, R., 2007. Nutritional upgrading for omnivorous carpenter ants by the endosymbiont *Blochmannia*. BMC Biol. 5, 48.

Foster, J., Ganatra, M., Kamal, I., Ware, J., Makarova, K., Ivanova, N., Bhattacharyya, A., Kapatral, V., Kumar, S., Posfai, J., Vincze, T., Ingram, J., Moran, L., Lapidus, A., Omelchenko, M., Kyrpides, N., Ghedin, E., Wang, S., Goltsman, E., Joukov, V., Ostrovskaya, O., Tsukerman, K., Mazur, M., Comb, D., Koonin, E., Slatko, B., 2005. The *Wolbachia* genome of *Brugia malayi*: endosymbiont evolution within a human pathogenic nematode. PLoS Biol. 3, e121.

Gil, R., Belda, E., Gosalbes, M.J., Delaye, L., Vallier, A., Vincent-Monégat, C., Heddi, A., Silva, F., Moya, A., Latorre, A., 2008. Massive presence of insertion sequences in the genome of SOPE, the primary endosymbiont of the rice weevil *Sitophilus oryzae*. Int. Microbiol. 11, 41–48.

Gil, R., Latorre, A., Moya, A., 2004. Bacterial endosymbionts of insects: insights from comparative genomics. Environ. Microbiol. 6, 1109–1122.

Gil, R., Sabater-Munoz, B., Pérez-Brocal, V., Silva, F.J., Latorre, A., 2006. Plasmids in the aphid endosymbiont *Buchnera aphidicola* with the smallest genomes. A puzzling evolutionary story. Gene 370, 17–25.

Gil, R., Silva, F.J., Zientz, E., Delmotte, F., Gonzalez-Candelas, F., Latorre, A., Rausell, C., Kamerbeek, J., Gadau, J., Hölldobler, B., van Ham, R.C., Gross, R., Moya, A., 2003. The genome sequence of *Blochmannia floridanus*: comparative analysis of reduced genomes. Proc. Natl. Acad. Sci. USA 100, 9388–9393.

Gosalbes, M.J., Lamelas, A., Moya, A., Latorre, A., 2008. The striking case of tryptophan provision in the cedar aphid *Cinara cedri*. J. Bacteriol. 190, 6026–6029.

Heddi, A., Charles, H., Khatchadourian, C., Bonnot, G., Nardon, P., 1998. Molecular characterization of the principal symbiotic bacteria of the weevil *Sitophilus oryzae*: a peculiar G+C content of an endocytobiotic DNA. J. Mol. Evol. 47, 52–61.

Heddi, A., Grenier, A.M., Khatchadourian, C., Charles, H., Nardon, P., 1999. Four intracellular genomes direct weevil biology: nuclear, mitochondrial, principal endosymbiont, and *Wolbachia*. Proc. Natl. Acad. Sci. USA 96, 6814–6819.

Klasson, L., Walker, T., Sebaihia, M., Sanders, M.J., Quail, M.A., Lord, A., Sanders, S., Earl, J., O'Neill, S.L., Thomson, N., Sinkins, S.P., Parkhill, J., 2008. Genome evolution of *Wolbachia* strain wPip from the *Culex pipiens* group. Mol. Biol. Evol. 25, 1877–1887.

Klasson, L., Westberg, J., Sapountzis, P., Näslund, K., Lutnaes, Y., Darby, A.C., Veneti, Z., Chen, L., Braig, H.R., Garrett, R., Bourtzis, K., Andersson, S.G.E., 2009. The mosaic genome structure of the *Wolbachia* wRi strain infecting *Drosophila simulans*. Proc. Natl. Acad. Sci. USA 106, 5725–5730.

Kono, M., Koga, R., Shimada, M., Fukatsu, T., 2008. Infection dynamics of coexisting beta- and gamma-proteobacteria in the nested endosymbiotic system of mealybugs. Appl. Environ. Microbiol. 74, 4175–4184.

Lai, C.Y., Baumann, L., Baumann, P., 1994. Amplification of trpEG: adaptation of *Buchnera aphidicola* to an endosymbiotic association with aphids. Proc. Natl. Acad. Sci. USA 91, 3819–3823.

Lamelas, A., Pérez-Brocal, V., Gómez-Valero, L., Gosalbes, M.J., Moya, A., Latorre, A., 2008. Evolution of the secondary symbiont "*Candidatus Serratia* symbiotica" in aphid species of the subfamily Lachninae. Appl. Environ. Microbiol 74, 4236–4240.

Lefèvre, C., Charles, H., Vallier, A., Delobel, B., Farrell, B., Heddi, A., 2004. Endosymbiont phylogenesis in the Dryophthoridae weevils: Evidence for bacterial replacement. Mol. Biol. Evol. 21, 965–973.

López-Sánchez, M.J., Neef, A., Patiño-Navarrete, R., Navarro, L., Jiménez, R., Latorre, A., Moya, A., 2008. *Blattabacteria*, the endosymbionts of cockroaches, have a small genome sizes and high genome copy numbers. Environ. Microbiol. 10, 3417–3422.

López-Sánchez, M.J., Neef, A., Peretó, J., Patiño-Navarrete, R., Pignatelli, M., Latorre, A., Moya, A., 2009. Evolutionary convergence and nitrogen metabolism in *Blattabacterium* strain Bge, primary endosymbiont of the cockroach *Blattella germanica*. PLoS Genet. 5(11), e1000721, doi:10.1371/journal.pgen.1000721.

Matthew, C.Z., Darby, A.C., Young, S.A., Hume, L.H., Welburn, S.C., 2005. The rapid isolation and growth dynamics of the tsetse symbiont *Sodalis glossinidius*. FEMS Microbiol. Lett. 248, 69–74.

McCutcheon, J.P., Moran, N.A., 2007. Parallel genomic evolution and metabolic interdependence in an ancient symbiosis. Proc. Natl. Acad. Sci. USA 104, 19392–19397.

McCutcheon, J.P., McDonald, B.R., Moran, N.A., 2009a. Convergent evolution of metabolic roles in bacterial co-symbionts of insects. Proc. Natl. Acad. Sci. USA 106, 15394–15399.

McCutcheon, J.P., McDonald, B.R., Moran, N.A., 2009b. Origin of an alternative genetic code in the extremely small and GC-rich genome of a bacterial symbiont. PLoS Genet. 7, e1000565.

McMeniman, C.J., Lane, R.V., Cass, B.N., Fong, A.W., Sidhu, M., Wang, Y.F., O'Neill, S.L., 2009. Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. Science 323, 141–144.

Moran, N.A., 2007. Symbiosis as an adaptative process and source of phenotypic complexity. Proc. Natl. Acad. Sci. USA 104, 8627–8633.

Moran, N.A., Degnan, P.H., Santos, S.R., Dunbar, H.E., Ochman, H., 2005. The players in a mutualistic symbiosis: insects, bacteria, viruses, and virulence genes. Proc. Natl. Acad. Sci. USA 102, 16919–16926.

Moran, N.A., McCutcheon, J.P., Nakabachi, A., 2008. Genomics and evolution of heritable bacterial symbionts. Annu. Rev. Genet. 42, 165–190.

Moran, N.A., McLaughlin, H.J., Sorek, R., 2009. The dynamics and time scale of ongoing genomic erosion in symbiotic bacteria. Science 16, 379–382.

Moya, A., Peretó, J., Gil, R., Latorre, A., 2008. Learning how to live together: genomic insights into prokaryote–animal symbioses. Nat. Rev. Genet. 9, 218–229.

Munson, M.A., Baumann, P., Kinsey, M.G., 1991. *Buchnera* gen. nov. and *Buchnera aphidicola* sp. nov., a taxon consisting of the mycetocyte-associated, primary endosymbionts of aphids. Int. J. Syst. Bacteriol. 41, 566–568.

Nakabachi, A., Yamashita, A., Toh, H., Ishikawa, H., Dunbar, H.E., Moran, N.A., Hattori, M., 2006. The 160-kilobase genome of the bacterial endosymbiont *Carsonella*. Science 314, 267.

Nogge, G., 1976. Sterility in tsetse flies (*Glossina morsitans* Westwood) caused by loss of symbionts. Experientia 32, 995–996.

Nováková, E., Václav, H., Moran, N.A., 2009. *Arsenophonus*, an emerging clade of intracellular symbionts with a broad host distribution. BMC Microbiol. 9, 143–157.

Oliver, K.M., Degnan, P.H., Burke, G.R., Moran, N.A., 2009. Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. Annu. Rev. Entomol. 55, 247–266.

Plague, G.R., Dunbar, H.E., Tran, P.L., Moran, N.A., 2007. Extensive proliferation of transposable elements in heritable bacterial symbionts. J. Bacteriol. 190, 777–779.

Pérez-Brocal, V., Gil, R., Ramos, S., Lamelas, A., Postigo, M., Michelena, J.M., Silva, F.J., Moya, A., Latorre, A., 2006. A small microbial genome: The end of a long symbiotic relationship? Science 314, 312–313.

Redak, R.A., Purcell, A.H., Lopes, J.R., Blua, M.J., Mizell, R.F., Andersen, P.C., 2004. The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. Annu. Rev. Entomol. 49, 243–270.

Rio, R.V.M.T, Lefebvre, C., Heddi, A., Aksoy, S., 2003. Comparative genomics of insect-symbiotic bacteria: influence of host environment on microbial genome composition. Appl. Environ. Microbiol. 69, 6825–6832.

Russell, J.A., Latorre, A., Sabater-Muñoz, B., Moya, A., Moran, N.A., 2003. Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea. Mol. Ecol. 12, 1061–1075.

Russell, J.A., Moran, N.A., 2005. Horizontal transfer of bacterial symbionts: heritability and fitness effects in a novel aphid host. Appl. Environ. Microbiol. 71, 7987–7994.

Sabree, Z.L., Kambhampatic, S., Moran, N.A., 2009. Nitrogen recycling and nutritional provisioning by *Blattabacterium*, the cockroach endosymbionts. Proc. Natl. Acad. Sci. USA 106, 19521–19526.

Sassera, D., Beninati, T., Bandi, C., Bouman, E.A.P., Sacchi, L., Fabbi, M., Lo, N., 2006. '*Candidatus* Midichloria mitochondrii', an endosymbiont of the tick *Ixodes ricinus* with a unique intramitochondrial lifestyle. Int. J. Syst. Evol. Microbiol. 56, 2535–2540.

Sassera, D., Lo, N., Bouman, E.A.P., Epis, S., Mortarino, M., Bandi, C., 2008. "*Candidatus* Midichloria" endosymbionts bloom after the blood meal of the host, the hard tick *Ixodes ricinus*. Appl. Environ. Microbiol. 74, 6138–6140.

Salzberg, S.L., Puiu, D., Sommer, D.D., Nene, V., Lee, N.H., 2009. Genome sequence of the *Wolbachia* endosymbiont of *Culex quinquefasciatus* JHB. J. Bacteriol. 191, 1725.

Schmitt, S., Wehrl, M., Bayer, K., Siegl, A., Hentschel, U., 2007. Marine sponges as models for commensal microbe-host interactions. Symbiosis 44, 43–50.

Shigenobu, S., Watanabe, H., Hattori, M., Sakaki, Y., Ishikawa, H., 2000. Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. Nature 407, 81–86.

Tamames, J., Gil, R., Latorre, A., Peretó, J., Silva, F.J., Moya, A., 2007. The frontier between cell and organelle: Genome analysis of *Candidatus* Carsonella ruddii. BMC Evol. Biol. 7, 181.

Tamas, I., Klasson, L., Canbäck, B., Näslund, A.K., Eriksson, A., Wernegreen, J.J., Sandström, J.P., Moran, N.A., Andersson, S.G.E., 2002. 50 million years of genomic stasis in endosymbiotic bacteria. Science 296, 2376–2379.

Thao, M.L., Moran, N.A., Abbot, P., Brennan, E.B., Burckhardt, D.H., Baumann, P., 2000. Cospeciation of psyllids and their primary prokaryotic endosymbionts. Appl. Environ. Microbiol. 66, 2898–2905.

Toft, C., Fares, M.A., 2008. The evolution of the flagellar assembly pathway in endosymbiotic bacterial genomes. Mol. Biol. Evol. 25, 2069–2076.

Toh, H., Weiss, B.L., Perkin, S.A., Yamashita, A., Oshima, K., Hattori, M., Aksoy, S., 2006. Massive genome erosion and functional adaptations provide insights into the symbiotic lifestyle of *Sodalis glossinidius* in the tsetse host. Genome Res. 16, 149–156.

Tremblay, E., 1989. Coccoidea endosymbiosis. In: Schwemmler, W., Gassner, G. (Eds.), Insect Endocytobiosis: Morphology, Physiology, Genetics, Evolution. CRC Press, Boca Raton, pp. 145–173.

Turley, A.P., Moreira, L.A., O'Neill, S.L., McGraw, E.A., 2009. *Wolbachia* infection reduces blood-feeding success in the dengue fever mosquito, *Aedes aegypti*. PLoS Negl. Trop. Dis. 3, e516.

van Ham, R.C., Kamerbeek, J., Palacios, C., Rausell, C., Abascal, F., Bastolla, U., Fernandez, J.M., Jimenez, L., Postigo, M., Silva, F.J., Tamames, J., Viguera, E., Latorre, A., Valencia, A., Moran, F., Moya, A., 2003. Reductive genome evolution in *Buchnera aphidicola*. Proc. Natl. Acad. Sci. USA 100, 581–586.

Van Ham, R.C., Martínez-Torres, D., Moya, A., Latorre, A., 1999. Plasmid-encode danthranilate synthase (TrpEG) in *Buchnera aphidicola* from aphids of the family Pemphigidae. Appl. Environ. Microbiol. 65, 117–125.

Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A., Wu, D.Y., Paulsen, I., Nelson, K.E., Nelson, W., Fouts, D.E., Levy, S., Knap, A.H., Lomas, M.W., Nealson, K., White, O., Peterson, J., Hoffman, J., Parsons, R., Baden-Tillson, H., Pfannkoch, C., Rogers, Y., Smith, H.O., 2004. Environmental genome shotgun sequencing of the Sargasso Sea. Science 304, 58–60.

Wu, D., Daugherty, S.C., van Aken, S.E., Pai, G.H., Watkins, K.L., Khouri, H., Tallon, L.J., Zaborsky, J.M., Dunbar, H.E., Tran, P.L., Moran, N.A., Eisen, J.A., 2006. Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. PLoS Biol. 4, e188.

Wu, M., Sun, L.V., Vamathevan, J., Riegler, M., Deboy, R., Brownlie, J.C., McGraw, E.A., Martin, W., Esser, C., Ahmadinejad, N., Wiegand, C., Madupu, R., Beanan, M.J., Brinkac, L.M., Daugherty, S.C., Durkin, A.S., Kolonay, J.F., Nelson, W.C., Mohamoud, Y., Lee, P., Berry, K., Young, M.B., Utterback, T., Weidman, J., Nierman, W.C., Paulsen, I.T., Nelson, K.E., Tettelin, H., O'Neill, S.L., Eisen, J.A., 2004. Phylogenomics of the reproductive parasite *Wolbachia pipientis* wMel: a streamlined genome overrun by mobile genetic elements. PLoS Biol. 2, e69.

Xie, G., Bruce, D.C., Challacombe, J.F., Chertkov, O., Detter, J.C., Gilna, P., Han, C.S., Lucas, S., Misra, M., Myers, G.L., Richardson, P., Tapia, R., Thayer, N., Thompson, L.S., Brettin, T.S., Henrissat, B., Wilson, D.B., McBride, M.J., 2007. Genome sequence of the cellulolytic gliding bacterium *Cytophaga hutchinsonii*. Appl. Environ. Microbiol. 73, 3536–3546.

Zientz, E., Dandekar, T., Gross, R., 2004. Metabolic interdependence of obligate intracellular bacteria and their insect hosts. Microbiol. Mol. Biol. Rev. 68, 745–770.