Symbionts and Pathogens: What is the Difference?

Vicente Pérez-Brocal, Amparo Latorre and Andrés Moya

Abstract The ecological relationships that organisms establish with others can be considered as broad and diverse as the forms of life that inhabit and interact in our planet. Those interactions can be considered as a continuum spectrum, ranging from beneficial to detrimental outcomes. However, this picture has revealed as more complex and dynamic than previously thought, involving not only factors that affect the two or more members that interact, but also external forces, with chance playing a crucial role in this interplay. Thus, defining a particular symbiont as mutualist or pathogen in an exclusive way, based on simple rules of classification is increasingly challenging if not unfeasible, since new methodologies are providing more evidences that depict exceptions, reversions and transitions within either side of this continuum, especially evident at early stages of symbiotic associations. This imposes a wider and more dynamic view of a complex landscape of interactions.

V. Pérez-Brocal (✉)
Área de Genómica y Salud, Centro Superior de Investigación en Salud Pública (CSISP), Avenida de Cataluña 21, 46020 Valencia, Spain
e-mail: perez_vicbro@gva.es

A. Latorre
CIBER en Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain

A. Moya
Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, Apartado Postal 22085, 46071 Valencia, Spain

Current Topics in Microbiology and Immunology (2013) 358: 215–243
DOI: 10.1007/82_2011_190

Published Online: 11 November 2011

Contents

1 Introduction

All forms of life on Earth interact with their environment that surrounds them. This includes abiotic factors to which organisms respond as well as other living organisms, from the same or different species. Within the enormous diversity of living beings on Earth, one of the groups that undoubtedly have achieved higher success and prosperity is that of the prokaryotes. A feature that characterizes them is their broad spectrum of metabolic capabilities. This has allowed them to colonize virtually the entire planet, from several kilometers inside the lithosphere to the troposphere, and live in all types of terrestrial and aquatic ecosystems, from the most favorable to the most extreme. No wonder, then, that many bacteria have developed physical partnerships with other organisms with more limited metabolic capabilities, such as other bacteria, protozoa, fungi, plants or animals. Their metabolic capabilities allow them to interact with eukaryotes to exploit their resources enabling bacteria to spread at expenses of the eukaryotes, occasionally provoking harm (which, if produces clinical symptoms is called disease) or using them in more than counterbalanced fashion by the contribution of new metabolic capabilities or other benefits for the host. Such beneficial cooperation has enabled eukaryotic organisms and their prokaryotic partners to occupy new ecological niches and diversify in order to be more successful.

The term symbiosis which means 'living together' designates the interdependence of two (or more) organisms of different species that results in a benefit to all of the implied partners (mutualism), or just to some of them. In this case, it can involve harm of one of the species, (parasitism) or not (commensalism). This rather simplistic classification of the symbiosis in discrete categories is being challenged nowadays by a more complex and dynamic view of interaction within a continuum. The spectrum between mutualism and parasitism is continuous and it is often difficult to distinguish one from another since a single association may have positive or negative depending on the environmental circumstances.

The partners that establish a symbiotic association are called host and symbiont. The host organism is defined as the provider of resources or the resource base, while the symbionts are the consumers of such resources, and may or may not provide services in return. This is similar to the definition of Ferrière et al. (2007) who considered the host as the producer of commodities and the symbiont or

partner as provider of goods and services. When originally defined by Anton de Bary and Simon Schwendener in 1879, the terms symbiosis and symbiont did not considered whether the effects of the association were beneficial or detrimental for the partners. However, many authors have used these terms in a more restricted way as synonyms of mutualism and mutualist. In order to reduce this ambiguity, throughout this review we use the term symbiosis in the comprehensive meaning to include all kind of interactions (i.e. mutualism, parasitism, commensalism) and only refer to mutualism and mutualists when a benefit to the host is observed.

Symbiosis has acted in the past and still does as a major catalyst of evolution, contributing to the promotion of speciation, diversification and evolutionary novelties such as the development of cell types, tissues and organs to harbor mutualists as well as barriers against pathogens, including the development of the immune system. A classic example of the evolutionary role of symbioses is the endosymbiotic theory (Margulis 1981, 1993) that postulates an initial invasion of the ancestor of the eukaryotic cell by Alphaproteobacteria with a large capacity to consume oxygen, from which the mitochondria would eventually arise, followed by a second colonization by prokaryotes with chlorophyll, believed to be similar to cyanobacteria that gave rise to chloroplasts, resulting in photosynthetic cells such as plants, which have both mitochondria and chloroplasts.

The application of recent technological developments (next-generation sequencing, metagenomics and synthetic biology) has allowed a significant progress in making possible the study of environmental samples and non-cultivable microorganisms, thus offering new opportunities for deciphering the associations between microorganisms and their hosts as well as among different microorganisms within hosts. For example; whole genome amplification methods facilitate genomic studies of host-associated bacteria for which only limited amounts of DNA are available. Metagenomics may reveal how genes interact in bacterial consortia that inhabit the same hosts and tissues and how these interactions affect the outcome of the infection. And synthetic biology holds promise in providing tools to study the function of genes under non-conventional or controlled experimental conditions (Toft and Andersson 2010).

Some examples of symbiotic interactions between bacteria and different eukaryotic hosts are shown in Table 1. A great number of mutualistic interactions have a nutritional character, particularly well studied in arthropods with specialized diets such as sap-sucking, blood-sucking or grain-feeding insects, where bacterial mutualists provide them with essential amino acids and/or vitamins and cofactors. But they can also exert other metabolic roles, in nitrogen recycling, storage and excretion as in omnivorous insects or clams. Finally benefits to the host can be more environmentally related, such as protection against parasitoids, predators and abiotic stress or other functions such as luminescence used by many animals for communication, attraction and other behaviors. On the other hand, the parasitism develops for example between pathogenic bacteria and animals, plants or fungi, causing infectious diseases, reproductive alterations and lesions that can produce the death of the infected host.

Table 1.1 Some examples of bacteria-host symbiotic interactions

Type of interaction	Prokaryotic Partner	Host Partner	Proposed benefit/harm	Type of interaction	Prokaryotic Partner	Host Partner	Proposed benefit/harm
M	*Symbionidium spp.*	Corals	Nutrient provision	P	*Holospora spp.*	Paramecium	Cell death
M	*Hamiltonella defensa*[a]	Pea aphid	Resistance to parasitoid wasps	P	*Vibrio shiloi*	Coral	Bacterial bleaching
M	*Regiella insecticola*[a]	Pea aphid	Resistance to fungal pathogens	P	*Phormidium corallyticum*	Coral	Black band disease
M	*Serratia symbiotica* [a]	Cedar aphid	Nutrient provision	P	*Pasteuria penetrans*	Root knot nematode	Host sterilization
M	*Wolbachia pipientis*	Nematode	Nutrient provision	P	*Cardinium hertigii a*	Many arthropods	Reproductive parasite
M	*Blochmannia spp.*[a]	Carpenter ant	Nutrient provision-N storage	P	*Wolbachia pipientis*	Many arthropods	Reproductive parasite
M	*Wigglesworthia glossinidia*	Tsetse fly	Nutrient provision	P	*Serratia entomophila*	Grass grub	Septicemia and death
M	*Buchnera aphidicola*	Aphids	Nutrient provision	P	*Rhabdochlamydia crassificans*	Cockroach	Abdominal swelling
M	*Blattabacterium spp*	Cockroach	Nutrient provision-N excretion	P	*Rickettsia spp.*	Fleas, lices, ticks	Insect death
M	*Sulcia muelleri* [a]	Sharpshooter, cicada, spittlebug	Nutrient provision	P	*Photorhabdus spp.*	Many insects	Lethal septicemia
M	*Baumannia cicadellinicola*[a]	Sharpshooter	Nutrient provision	P	*Spiroplasma spp.*	Butterflies	Reproductive parasite
M	*Hodgkinia cicadicola*[a]	Cicada	Nutrient provision	P	*Pasteuria ramosa*	Daphnia	Host sterilization
M	*Zinderia insecticola*[a]	Spittlebug	Nutrient provision	P	*Aeromonas salmonicida*	Salmonids	Furunculosis
M	*"SOPE", "SZPE"*	Weevil	Nutrient provision	P	*Yersinia pestis*	Human	Bubonic plague
M	*Ruthia magnifica*[a]	Deep-sea clam	Nutrient provision-N recycling	P	*Vibrio cholerae*	Human	Cholera
M	*Vibrio fischeri*	Loliginid squids	Chemiluminescence	P	*Xylella fastidiosa*	Grapevine	Pierce's disease
M	*Rhizobium spp.*	Leguminous plants	Nitrogen fixation	P	*Xanthomonas spp.*	Various plants	Necrotic lesions

[a] These bacteria are called Candidatus. M: mutualism, P: parasitism

In this review, we address a number of features that characterize mutualistic symbionts and parasites to try to establish some criteria, if any, that define them, with the purpose of understanding what defines a bacterium as beneficial or harmful. In addition, we address the enormous diversity of interactions that they establish and the complexity that this implies when it comes to classify them.

2 The Plasticity of Symbiotic Associations

The boundaries of parasitism, commensalism and mutualism are vague. By considering the various forms of symbioses as existing throughout a theoretical continuum of fitness outcomes, it is possible to note that mutualism and parasitism may in fact be considered as two sides of the same coin. The symbiotic associations cannot be merely considered as closed-circuits isolated from the external circumstances. In fact, Leung and Poulin (2008) showed how easily symbiotic associations can switch between mutualism and parasitism in response to even the slightest environmental change. Actually, extrinsic factors such as environmental circumstances or time, as well as intrinsic factors such as the transmission mode, can influence the symbiotic relationship, determining that a priory beneficial outcome becomes detrimental if the circumstances change or vice versa.

One aspect to consider when studying symbiotic associations is represented by the fact that the costs and benefits of a symbiosis for a host organism are not always evident or easily measurable because they may exist on many levels (Cushman and Beattie 1991). Parasites may impose with their presence additional costs or mutualist partners may confer multiple benefits to their hosts beyond the immediate physiological effects. For example, bacterial mutualists that confer their hosts novel capabilities and therefore favor the exploitation of novel resources produce the niche expansion for the host, something that has in turn fitness benefits because the hosts find fewer competitors in those unexploited niches (Moran 2007). The pests caused by sap-feeding insects, such as aphids, psyllids, whiteflies, mealybugs, sharpshooters or cicadas illustrate the success of their associations with bacterial obligate endosymbionts. On the contrary, pathogens, in addition to the pathology usually associated with their infection per se, may impose changes in behavior that make them more vulnerable to predators, for example.

Costs of a symbiosis may be hard to detect, possibly even completely hidden due to the host's phenotypic plasticity. If the host eventually evolves to completely tolerate the parasitic infection as a way of mitigating the harm caused by a parasite, the result may superficially appear to be a commensalism when actually it has come about at a significant fitness cost to the host, which has since been masked over evolutionary time (Miller et al. 2006). This happens, for example with the attenuated pathogens, which can be the result of a long history and coevolution with their hosts. At first, bacteria from the environment but sometimes also from the endogenous microbiota can acquire from other strains or species external elements called factors of virulence that render them virulent and able

to provoke disease. Susceptible host populations, which are not initially adapted to these newly arisen pathogens, also known as emergent pathogens, may first lead to a dramatic infection and an epidemic. Once a pathogen persists in the new population, the disease tends to become less virulent with time because the hosts are enforced to develop mechanisms of defence able to fight against the interloper as in an "arms race". In this context, if pathogens and hosts coevolve, one possibility is that the pathogen attenuates itself, and eventually a balance is established with the host. This attenuation of the pathogen and increasing dependence on the host may even result in some cases in mutualistic relationships. A possible example of this extreme transition is found in *Wolbachia* spp. which has been described as a reproductive manipulator of many arthropods, but more recently evolved mutualistic relationships with the common bedbug *Cinex lecturalius*, to which it provides with vitamin B, have been discovered (Hosokawa et al. 2010). Even more, *Wolbachia* spp. was transformed in natural populations of *Drosophila simulans* in less than 25 years from a reproductive manipulator to a mutualist that enhanced fecundity (Weeks et al. 2007). Other possibilities are that the pathogen remains virulent or regains virulence due to genetic changes or genetic exchange via horizontal gene transfer leading to new epidemic episodes until the host again responds and another balance is reached. In all these cases, time plays as a key factor, and not only on an evolutionary scale, but even relative short time periods, as demonstrated in *Wolbachia's* example.

Besides the direct costs that pathogens cause to the host fitness in the form of infectious diseases, all parasites take resources from the host, also reducing host fitness in the process as a side effect. But the latter also applies to mutualists which also demand resources from their host. However this cost is usually compensated by the benefit simultaneously conferred on the host by the mutualistic symbiont. This raises the question of whether benefits to host fitness can be surpassed by the costs under certain environments and circumstances or vice versa. The balance between the costs and benefits for the two participants in a symbiosis depends on a range of factors, and often only a small push is needed to shift that balance. An example of the fact that the fitness costs of the association are not always counterbalanced by the benefits is found in the pea aphid *Acyrtosiphon pisum*, which in addition to the obligate endosymbiont *Buchnera aphidicola*, can also harbor a range of facultative secondary symbionts. One of them, "*Ca.* Hamiltonella defensa" was demonstrated to confer to its host resistance against the attack of parasitoid wasps (Oliver et al. 2005). However, aphids with secondary symbionts experience a severe fecundity reduction in comparison with uninfected aphids (Oliver et al. 2006). Symbiont-based fitness differentials are also known for "*Ca.* Serratia symbiotica" and "*Ca.* Regiella insecticola", which affect the ability to withstand heat (Russell and Moran 2006), to use particular host plants (Tsuchida et al. 2004), or to resist pathogenic fungi (Scarborough et al. 2005). Benefits in a natural environment, which can explain the maintenance of certain symbioses, may not be obvious under other conditions, experimental or natural, if the factors that facilitate its persistence are absent. For example, at temperatures above 30°C *B.aphidicola* is eliminated and bacteriocytes are reduced, resulting in the absence

of reproduction in aphids, whereas the presence of a secondary symbiont, in conditions of heat stress, resulted in an increase in the reproduction of the host (Montllor et al. 2002), which is also related to the degree of variation in the frequency of secondary symbionts in less stable climates (Haynes et al. 2003).

In addition to the extrinsic factors, an intrinsic key factor in determining the virulence of a symbiont is the mode of transmission (Ewald 1995; Day 2001; Ferdy and Godelle 2005). Thus a vertical transmission implies an alignment of the fitness outcomes of both the symbiont and the host. Cooperation between the two parties or at least lower virulence by the symbiont would be an outcome favored by selection (Ewald 1995). In contrast, if the fitness of the symbiont is not exclusively interweaved with that of its host, then its fitness can be improved by exploiting its host more aggressively while returning fewer benefits, which should push the association on the evolutionary path toward parasitism.

Hosts and bacterial symbionts play an active role in the income-outcome of the symbiotic associations. As for any kind of interaction, in both mutualistic and pathogenic associations there is a conflict of interests between the interacting partners. Thus, they should be viewed as an "arms race" in which changes in one partner must be matched by adaptive changes in the other to maintain equilibrium (Steinert et al. 2000). The need for mechanisms such as host resistance and immunological responses by the hosts has resulted from the conflict of interests that exists within any obligate biological association, and symbiont control and immunological responses perform the role of limiting harm or preventing overexploitation of the host by the symbionts in question. The only difference is that in the case of host−parasite interaction, the relationship is more antagonistic—the host is attempting to completely deny any resources to the symbiont, whereas the latter is attempting to exploit the host while returning nothing of benefit. In this arms raced, which are the weapons that hosts and symbionts brandish? From the host side, defence mechanisms against intruders encompass a broad and varied range of physical barriers (skin, cuticle, mucus, cilia, etc.) and complex immune reactions to cope with the invader. The latter evolved in complexity from simpler responses in the oldest extant metazoan phyla (Müller and Müller 2003) to the most sophisticated ones of vertebrates. The immune system has been particularly well studied in insects (Feldhaar and Gross 2008; Govind 2008; Strand 2008; Gerardo et al. 2010) and vertebrates (Male 2004; Pier et al. 2004; Meyers 2007). Insects rely chiefly on the innate immune system, although new findings challenge this assumption (Kurtz et al. 2006; Schulenburg et al. 2007). This is the oldest one in evolutionary terms, and can be divided into cellular responses, based on haemocytes in insects involving phagocytosis and encapsulation; and the humoral responses resulting in the production of soluble antimicrobial peptides, melanisation and clotting. Responses vary depending on the invader (e.g. antimicrobial peptides against microbes and encapsulation against parasitoids). In insect genomes, there are four pathways that appear well conserved (invasive microbe leads to signal production via four pathways (Toll, immunodeficiency (IMD), c-Jun N-terminal kinase (JNK), and Janus kinase/Signal transducers and activators of transcription (JAK/STAT)). Each pathway is activated in response to particular pathogens (Dionne and Schneider 2008). However, until recently, only

holometabolous insects (i.e. with complete metamorphosis) had been analyzed. The study of the immunity and stress in the recently sequenced genome of the hemimetabolous pea aphid *Acyrtosiphon pisum* (Gerardo et al. 2010), which is also the first intimately dependent on obligate and facultative bacterial symbionts for its survival, reveals that many genes central to immune function in other insects are missing (e.g. peptidoglycan receptor proteins, the IMD signaling pathway, defensins, c-type lysozymes) and thus the overall response is more limited. Forces such as reproductive investment to an immune challenge, rather than in a costly immune response, or symbiont-mediated host protection by secondary symbionts, could potentially shape the evolution of aphis stress and immune responses, although more studies characterizing the global aphid response under more conditions are needed. In superior vertebrates, in addition to the innate or nonspecific responses, which include nutritional immunity, the action of the complement and phagocytosis and opsonophagocytosis, an adaptive or specific immunity has developed. This response is based on the recognition of specific antigens by immunoglobulins, T cell receptors and major histocompatibility complex molecules (Davis and Bjorkman 1988; Lebecque and Bearhart 1990; Tonegawa 1983). It comprises humoral responses, mediated by the interaction of B and T helper cells implying the production of antibodies, and cellular responses, triggered by the interaction of macrophages and inflammatory T cells or any infected cell and cytotoxic T cells. Such reactions activate a series of mechanisms to fight the intruders, including the complement activation, toxins neutralization, agglutination reactions, blocking of adhesion of bacteria to epithelia, induction of the free O_2 radicals or liberation of cytotoxins to kill infected cells.

In order to overcome these host mechanisms but also to gain access to the nutrient-rich environment that the host represents the symbionts have developed a range of countermeasures. Since the biological processes needed to successfully infect hosts are largely the same for both types of microorganisms (Gil et al. 2004), some general strategies for symbiont's survival can be applied, no matter if the relationship becomes parasitic or mutualist. In all cases the first stage is the finding of a way of entering the host. Next, the symbionts must find unique niches within hosts. Third, it will be necessary to avoid, circumvent or subvert normal host cell defenses. The goal for the bacteria is to multiply sufficiently either to establish their progeny within the host and/or to move into a new susceptible host. A low infectious dose along with an efficient transmission tactic is a potent strategy for a microorganism to sustain its progeny. Genomic analyses indicate that many molecular factors (toxins, islands of pathogenicity, type III secretion systems, ureases, etc.) are involved both in pathogenic and mutualistic relationships. Toxins are virulence factors expressed by some pathogens to establish in a particular host and transmit to new susceptible hosts by causing direct harm to the so-called AG resines, (e.g. ADP-ribosylation toxins, neurotoxins, Rnase, cytotoxins, etc.) or indirect harm, the so-called modulines (e.g. lipopolysaccharide, superantigens, etc.) to the hosts. Examples of toxins include the diphtheria toxin, the botulimun toxin, the tetanus toxin or the cholera toxin. However, toxins may also play a role in mutualistic associations. For instance, facultative symbionts produce antifungal compounds and toxins that are known or

suspected to be involved in protecting the host against natural enemies. For example, symbionts produce antifungal metabolites that protect their crustacean hosts *Palaemon macrodactylus* and *Homarus americanus* from the pathogenic fungus *Lagenidium callinectes* (Gil-Turnes et al. 1989; Gil-Turnes and Fenical 1992). Similarly, possession of the facultative symbiont "*Ca.* Regiella insecticola" is associated with host resistance to a fungal pathogen *Pandora neoaphidis* in aphids (Ferrari et al. 2004; Scarborough et al. 2005) and "*Ca.* Serratia symbiotica" and "*Ca.* Hamiltonella defensa" confer protection to their aphid host from the parasitoid wasps *Aphidius ervi* and *Aphidius eadyi* (Oliver et al. 2003, 2005, 2009; Ferrari et al. 2004). Another element is that of genomic islands which are movable genetic elements located in the chromosome, flanked by specific sequences (direct repeats), associated with tRNA loci, and often possess genes encoding for genetic mobility (phages, IS elements, integrases, transposases, origins of replication). There are pathogenicity islands (e.g. PaiI and PaiII of E. coli, linked to resistance to blood serum and union to urinary epithelium, respectively), but more recently symbiosis islands (e.g. *Mesorhizobium loti* R7A symbiotic island, Sullivan et al. 2002) have also been reported.

Even endosymbiotic bacteria with a long-time established relationship with their hosts and dramatic genome size reduction maintain genes that encode essential endosymbiotic factors that are proposed to be virulence associated in bacterial pathogens, such as type III secretion systems and urease (Gil et al. 2003; Goebel and Gross 2001; Shigenobu et al. 2000). In many free-living bacteria, genes encoding the type III secretion system are located within pathogenicity islands that have been acquired by horizontal gene transfer. This system is present in many insect endosymbiotic bacteria where it has been proposed to be essential to invade the host cells, thus playing an essential role in the establishment of the symbiosis (Dale et al. 2001, 2002). In some pathogenic microorganisms, ureases have been identified as virulence factors (e.g. Rokita et al. 1998) whereas in the P-endosymbiont of carpenter ants, "*Ca.* Blochmannia", it has become beneficial in the recycling of nitrogen in phases such as the metamorphosis (Gil et al. 2003; Degnan et al. 2005).

Little is known about the molecular basis of interactions of mutualists-hosts in comparison to that of pathogens-hosts. The coexistence of these bacteria with the host without triggering major immune responses suggests no recognition of the bacteria or active evasion the immune system of the host. The genome sequences of several primary endosymbionts of insects show the loss of enzymes required for the biosynthesis of peptidoglycan and lipopolysaccharide (Zientz et al. 2004). This may indicate a reduction in that the visibility of the bacteria by the host immune system. In addition, their intracellular location may protect them from immune recognition and defense mechanisms. The recent characterization of the bacteriocyte transcriptome of the aphid *A. pisum* harboring the primary endosymbiont *B. aphidicola* revealed a specific up-regulation of genes involved in defense responses including those encoding putative lysozymes and may possibly function to control the endosymbiont population (Nakabachi et al. 2005). Some intracellular endosymbionts may also be found extracellularly at least during some phases of

the host's life cycle. For example, during metamorphosis the primary endosymbiont of the weevil *Sitophilus zeamais* (SZPE) migrates from the larval to the adult bacteriocytes situated around the foregut and close to the hindgut, respectively. As a reaction the host increases the expression of a peptidoglycan recognition protein (PGRP), possibly to avoid bacterial invasion into insect tissues other than the bacteriome (Anselme et al. 2006). In the case of the secondary endosymbiont of tsetse flies, *Sodalis glossinidius* found in the hemolymph and bacteriocytes, the host constitutively expresses high levels of the antimicrobial peptide Diptericin to which *S. glossinidius* is about ten times more resistant than *E. coli*. Thus, the endosymbiont may have adapted to the antimicrobial activity and/or the host attempts to control symbiont number by the constitutive expression of high levels of Diptericin (Hao et al. 2001).

3 What Do Pathogens and Symbionts Have in Common and What Separates Them?

Approaches from the Ecology, Immunology, Comparative Genomics and Biochemistry can provide us with some clues to understand similarities and differences between mutualists and pathogens. Thus, some traits shared by all symbionts, no matter the outcome of the symbiosis, illustrate the existence of convergences despite their apparently different lifestyles.

Mutualists and parasites share some common genetic and molecular features (Goebel and Gross 2001), to the point that they can be considered as two different results of a process that would begin similarly: with the encounter between host and symbiont, the infection and colonization of the host cells and tissues, the avoidance of the defense mechanisms and the persistence and multiplication within the host. As shown in Sect. 2, molecular factors (toxins, islands of pathogenicity, type III secretion systems, ureases …) are involved both in pathogenic and mutualistic relationships. In fact, the definition of pathogen given by Fallow 1997 as "any microorganism whose survival is dependent upon its capacity to replicate and persist on or within another species by actively breaching (…) a cellular or humoral host barrier that ordinarily restricts or inhibits other microorganisms" may apply with some nuances to mutualists, because it does not imply the final outcome for the host fitness, which is regarded as the main line that separates pathogens and mutualists. In this sense, the cardinal difference can be established between a commensal species and a pathogen or mutualistic one is that the latter has a capacity to break the host cell barriers to gain access to niches that commensals cannot. However, commensals may cause opportunistic infections if the host defense is breached.

Comparative genomic analysis of obligate intracellular bacteria has revealed a series of features shared by intracellular parasites and mutualistic endosymbionts, when compared with their free-living relatives. These include genome size reduction, almost total absence of recombination, increased rate of nucleotide

substitution, high A + T content, accumulation of deleterious mutations by random genetic drift, loss of codon bias toward A or T and accelerated sequence evolution (Moran 1996; Andersson and Kurland 1998; Clark et al. 1999; Moya et al. 2002). These genome features of obligate symbionts including elevated sequence evolution, gene loss and shift toward higher AT content (Dale and Maudlin 1999; McCutcheon and Moran 2007, Moya et al. 2002) are observed at less extreme levels in facultative symbionts.

Interestingly, there is a remarkable convergence in which genes have been lost from entirely free-living ancestors in the current obligate pathogens and mutualists. Both retain genes underlying the basic functions of cell growth and division, such as replication, transcription, translation and energy metabolism. On the other hand, lost loci include in both groups a large proportion of genes encoding DNA repair and recombination functions, although particular repair genes are retained and lost vary. Also, many genes required for production of cell envelope components are missing. Nevertheless, similarities end when we observe the metabolic capabilities retained by each type of symbiont. Small genome pathogenic bacteria have also lost genes for intermediate metabolism and biosynthetic pathways (Andersson and Kurland 1998; Fraser et al. 1995), observations congruent with the fact that many of the required metabolites are available in the nutrient-rich environment of the host cell. On the other hand, small genome obligate mutualists have retained many metabolic genes required for the biosynthesis of metabolites required by the host. For example genes required for the synthesis of essential amino acids (e.g. in *B. aphidicola*, "*Ca.* Sulcia muelleri", and SOPE) or vitamins and cofactors (e.g. in "*Ca.* Baumannia cicadellinicola" and "*Ca.* Hodgkinia cicadicola", SOPE and *Wigglesworthia glossinidia*) (Gosalbes et al. 2010).

Symbionts that form chronic infections share a common syndrome of genomic reduction and accelerated sequence evolution because they also share some of the population dynamics. They both reside in sequestered habitats where their reduced effective population size and reduced opportunities for recombination (by physical barriers and loss of recombinase genes) imply that the effectiveness of selection is decreased whereas the levels of genetic drift increase, resulting in the accumulation of mildly deleterious mutations, which unlike free-living bacteria, are not effectively eliminated by selection and thus they might fix by genetic drift. Bottlenecks are inherent to the mode of transmission of mutualists and pathogens, either due to the small number of bacteria transmitted maternally, where the host progeny receives only a limited diversity of bacteria present in their mother or, in horizontally transmitted pathogens (also in facultative symbionts), where each infection event implies a small number of bacteria passing to the new host. As a result of the accumulation of mildly deleterious mutations in this context of relaxed purifying selection plus reduced mechanisms of repair and recombination, gene inactivation is easily followed by gene erosion and loss, which is not balanced by gene uptake, unlike free-living bacteria. Unlike chronic pathogens and mutualists, which spend their life cycles closely associated with host cells, many other symbionts establish more labile interactions with eukaryotes. These so-called facultative or opportunistic symbionts may shift easily the type of association

through gene loss or acquisition via frequent horizontal transfer, and as long as they can grow as free-living bacteria, they can also grow to achieve large effective population sizes and retain metabolic capabilities necessary to grow as free-living bacteria. This results in a population dynamics notably different from obligate symbionts, and more similar to that of entirely free-living bacteria. Not surprisingly, these bacteria do not show the strong effect of drift or the genome reduction that characterizes obligate mutualists and pathogens.

Another hypothetically major difference between mutualists and pathogens that is blurred when we consider the whole spectrum of interactions is their mode of transmission. Obligate heritable mutualists such as the bacteriome-associated symbionts of insects display a mode of transmission strictly vertical, with a process starting at early stages of oogenesis or embryogenesis. This maternal transmission is controlled by the host, since the bacteria cannot invade naïve hosts and have evolved to be dependent on host-based mechanisms for transmission. This is demonstrated repeatedly by the codiversification of obligate mutualists and their hosts since the ancient acquisition in each case. On the contrary, pathogens can be more or less host-specific, but, unlike mutualists, they do not show a pattern of codiversification with their hosts since the mode of transmission is intrinsically horizontal, imposed by the own nature of this kind of association. Since there is a trade-off between fitness gained through increased among-host transmission (infectivity) and fitness lost through increased virulence, virulence of pathogens will increase with horizontal transmission, whereas with vertical transmission, virulence will decrease because a parasite's reproductive potential will be maximized only by decreasing harm to the host, allowing parasite transmission to more host offspring (Bull et al. 1991; Agnew and Koella 1997; Stewart et al. 2005). In other words, as pathogens become more harmful and decrease the reproduction rate of their host, they are less likely to be passed on to the hosts' offspring since there will be fewer offspring. Actually, as shown in Sect. 2, the mode of transmission plays a key role in determining the virulence of a symbiont (Ewald 1995; Day 2001; Ferdy and Godelle 2005). To complicate things further, facultative symbionts resemble obligate and opportunistic pathogens in that their successful persistence in their hosts rely on their own mechanisms and capabilities for invading new hosts, entering cells and tissues and countering host immune responses. There are observations, such as variation in their presence within or among individuals or species, coexistence of several lineages within a host or the presence of the same facultative symbiont in non-related hosts, which support the presence of horizontal transfer in these bacteria within and between species (O'Neill et al. 1992; Sandström et al. 2001). Also, phylogenetic evidence suggests that secondary mutualistic symbionts have undergone horizontal transfers. This occurs when the phylogenies of endosymbionts and their hosts are not congruent (Russell et al. 2003; Sandström et al. 2001). For example, fungus-growing ants have a mutualism with bacteria that produce antibiotics with activity against *Escovopsis*, a specialized microparasite capable of rapidly devastating the fungus garden. These mutualistic bacteria from the genus *Pseudonocardia* are primarily vertically transmitted by colony-founding queens. However, strict cocladogenesis

between the ants and the bacteria is disrupted, especially at the finer phylogenetic levels, by *Pseudonocardi*a strains switching between ant species, both within and between genera. Poulsen et al. (2005) found evidence for frequent *Pseudonocardia* switches between sympatric ant species within the genus *Acromyrmex*, indicating that horizontal symbiont transmission occurs and may be common. However, this transfer is not often observed in laboratory experiments. On the other hand, *Wolbachia* is a maternally inherited intracellular reproductive parasite of arthropods that distorts the sex ratio in host's progeny. It imposes fitness costs on its host, such as physiological impairment (Fleury et al. 2000), decreased sperm quality (Champion de Crespigny and Wedell 2006), reduced immune response (Fytrou et al. 2006), mortality of male embryos (Hurst et al. 1999a; Zeh and Zeh 2006) and reproductive failure resulting from cytoplasmic incompatibility (Perrot-Minnot et al. 2002). Thus, it is a parasite vertically transmitted. Furthermore, it has also developed long-term associations with nematodes (Langworthy et al. 2000; Werren et al. 2008) and even with other arthropods (Hosokawa et al. 2010). Also, Rickettsia comprises pathogens of vertebrates, reproductive manipulators of arthropods and bacteriocyte-associated mutualists, having been suggested that *Rickettsia* species have exchanged a putative maternal inheritance in arthropods for mammal that serve as new "vectors" of transmission between arthropod hosts (Darby et al. 2007). Thus, exceptions to the rule of mutualists vertically transmitted and parasites horizontally transmitted are found in both sides.

Phylogenetic placement of bacterial pathogens and mutualists suggests that bacterial symbionts that form chronic infections in animals belong to deeply branching clades that are strictly parasitic or strictly mutualistic (Moran and Wernegreen 2000). However, the more genomes are available and more the effect of the symbionts on their hosts are known, the tree becomes more intricate as different categories of symbionts according to their effects fall into the same or closely related clades (Ecker et al. 2005; Moran et al. 2008). An increasing number of exceptions are challenging the classical trees as the isolated exceptions become more abundant. For instance, *Wolbachia* is a reproductive manipulator of arthropods (see above) but apparently a mutualist in filarial worms; the Flavobacteria contain obligate mutualists of cockroaches (*Blattabacterium*) as well as obligate pathogens of trouts and salmons (*Flavobacterium psychrophilum*); or Spirochaetes, which contain chronic pathogens (*Borrelia* spp.) but also mutualists of oligochaete worms, *Nautilus* and protozoa from termites. Moreover, the fact that certain groups concentrate more symbionts of either type (e.g. Gammaproteobacteria especially Enterobacteriaceae, and the Bacteroidetes represent an overwhelming majority within the obligate insect symbionts) could result simply from our limited knowledge and far-away from exhaustive sampling rather than a representative picture of the actual diversity of those symbionts. For example, newly characterized insect endosymbionts other than Gammaproteobacteria are being added to the existing ones (for example the Alphaproteobacterium "*Ca.* Midichloria mitochondrii" (Sassera et al. 2006), "*Ca.* Hodgkinia cicadicola" (Wu et al. 2006; McCutcheon et al. 2009), or the Betaproteobacterium "*Ca.* Zinderia insecticola" (McCutcheon and Moran 2010).

4 Classification of Symbioses: Different Strokes for Different Folks

In view of the fact that so many factors intermingle, when trying to define what pathogens and mutualists are, the criterion of choice determines different ways to classify the symbionts. On the one hand, there are "static" classifications based upon ecological principles. On the other hand, symbionts can be classified from a genomic point of view, with more dynamic evolutionary and temporal implications. In the first classification, factors such as the contribution to the host fitness, degree of dependence, location or specificity are contemplated. Thus, symbiotic associations have been generally divided into three categories based on whether the symbiont has beneficial, harmful or no effect on the host. When both partners reciprocally benefit from the relationship, the association represents mutualism, whereas if the symbiont uses the host as a resource and causes it harm as a result, it is qualified as a parasite. Finally, if the symbiont utilizes the host without benefiting or harming it, it is considered as a commensal, which might be regarded as a hodgepodge that reflects our ignorance of the effects of the symbiont on the host. Also, according to the degree of dependence of the symbiont (in the case of parasites) or both partners (in the case of mutualists), symbionts can be classified as obligate, primary or P-symbionts which only can be isolated from their host even if sporadically and during short periods some of them can be found in other environments. Their long-term survival absolutely depends upon its ability to replicate and to be transmitted in a particular host. In the case of mutualists of many invertebrates and in particular of insects, bacteria are typically restricted to a specialized organ called bacteriome, which varies depending on the host group, and are vertically or maternally inherited by the offspring. Primary pathogens cause disease in healthy individuals, with immune defenses unaltered. For example, *Bordetella pertussis* the etiological agent of the whooping cough. A special case is that of the co-primary symbionts (Wu et al. 2006; McCutcheon and Moran 2007). In this association or consortium, the symbionts coexist in the same host, being both essential for its survival. In contrast, the so-called opportunistic, facultative, secondary or S-symbionts show the capacity to survive outside of their natural hosts for long periods of time, and sometimes they have a natural habitat other than the host. Their long-term survival does not depend upon a particular host. They are usually more erratically distributed, with differences in their presence among populations and species, instances of co-infections with two or more of them in the same host, etc. In the case of mutualists of insects, these bacteria may invade various cell types and also reside extracellularly, and although vertical transmission is also common, they can also colonize novel hosts through horizontal transmission among host individual belonging to the same or different species (Dale and Moran 2006). Opportunistic pathogens cause disease only in individuals who are compromised in their immune defenses. For example, *Clostridium difficile* is a commensal bacterium of the intestine in a 2−5% of the human population which normally does not result in significant disease, but the introduction of broad-spectrum antibiotics causes disruption of normal intestinal flora, leading to an

overgrowth of *C. difficile* and diarrhea and severe infection of the colon leading to a disease called pseudomembranous colitis. This is an emergent nosocomial infection especially in hospitalized patients, who have a higher risk of contracting it. A third factor considered in this ecological classification is the location of the symbiont on or within the host. Those who live on the surface of the host, including the skin, gut, respiratory or genitourinary tract, or in the blood or hemolymph are called ecto-symbionts or extracellular symbionts. They can adhere and multiply on the cells but they do not invade epithelial barriers. Others have developed mechanisms of invasion and can be internalized by the eukaryotic cells, where they can remain transitorily in transit to their final niche or where they become residents. These are the so-called facultative or obligate endosymbionts or intra-cellular symbionts, respectively. In this case, the bacteria can be more or less integrated within the cell (in vacuoles or phagosomes, in the cytoplasm or even in the nucleus). For example, the pathogens *Legionella pneumophila*, *Chlamydia* spp., and *Mycobaterium* spp, or the mutualists *B. aphidicola*, and *Blattabacterium* spp. are harbored in vacuoles, whereas the pathogens *Listeria monocytogenes* or *Shigella* spp. and the mutualists "*Ca*. Blochmannia floridanus" and *Wigglesworthia glossinidia* live free in the cytosol. Finally the pathogen Rickettsia rickettsii has the ability to grow within the cytoplasm and the nuclei of their host cells (Silverman and Bound 1984). Finally, the symbiosis can be more host-specific or restricted to a single strain, species or genus, or alternatively, the symbiont can infect different non-related hosts. For example, *B. aphidicola* has been found exclusively in aphids, whereas "*Ca*. Sulcia muellerii" is found in sharpshooters, cicadas and spittlebugs. Another example, *Salmonella enterica* serovar Typhi can affect humans only, whereas serovar Thyphimurium has a broad range of hosts that include mice (Edwards et al. 2002).

The second perspective conceives the symbiosis from an evolutionary and temporal point of view, focusing on the transformations that affect the bacterial genomes from the establishment and the early stages of the symbiotic relationships to the final stages. A series of changes arise in both symbionts and hosts as a result of an adaptive process. This classification has been developed particularly well in the case of heritable bacterial symbionts of arthropods, where a quite complete picture of the process is being made available. The path from facultative symbiosis to early obligate endosymbiosis first, and toward long-established primary endosymbioses and the final stages in endosymbiotic relationships have been extensively reviewed in mutualistic symbioses between prokaryote and arthropods (Gil et al. 2010), but some of their principles may also be valid for other mutualistic and even parasitic symbioses. At the very beginning, it is not possible to determine if the relationship that would be established will be parasitic or mutualistic, since this distinction is based on the effect of the bacterium in the eukaryotic host but, from the bacterial point of view, the biological processes needed to successfully infect hosts are largely the same for both types of microorganisms (Gil et al. 2004).

Facultative or S-symbionts have been described in several lineages of aphids ("*Ca*. Hamiltonella defensa", "*Ca*. Regiella insecticola", and "*Ca*. Serratia symbiotica"), psyllids ("*Ca*. Arshenophonus"), whiteflies ("*Ca*. Hamiltonella", "*Ca*. Arsenophonus", "*Ca*. Cardinium", *Wolbachia*, *Rickettsia*, and *Fritschea*

bemisiae), leafhoppers (*Wolbachia*), scale insects (*Fritschea*, "*Ca*. Cardinium"), tsetse flies (*S. glossinidius*), fruit flies (*Spiroplasma*, *Wolbachia pipientis* wRi and wMel), hippoboscid flies (*Sodalis*), parasitic wasps ("*Ca*. Cardinium"), butterflies (*Spiroplasma*) and mosquitoes (*Wolbachia pipientis* wPip). (Moran et al. 2005a; Hansen et al. 2007; Gottlieb et al. 2008; Mitsuhashi et al. 2002; Everett et al. 2005; Zchori-Fein and Perlman 2004; Toh et al. 2006; Williamson and Poulson 1979; Stouthamer et al. 1999; Nováková and Hypsa 2007; Klasson et al. 2009; Jiggins et al. 2000; Klasson et al. 2008). Although they are generally maternally transmitted through host generations, their distribution patterns suggest sporadic horizontal transmission events among host individuals and species (Russell et al. 2003; Russell and Moran 2005), and, therefore they do not share long evolutionary histories with their hosts. In addition, their phylogeny points to relatively recent associations with their hosts (Dale and Moran 2006), unlike primary symbionts. Their genome traits may resemble those in the early stages of a transition from a free-living lifestyle to an obligate mutualism. S-symbionts show a wide distribution in tissues and cells or in the body cavity (hemolymph), sheath cells or specialized cells and organs called secondary bacteriocytes and bacteriomes, respectively (Fukatsu et al. 2000; Moran et al. 2005b), that are infected through their own mechanisms, rather than host adaptations. Their irregular distribution among individual hosts and species suggests that they are not essential for host survival, but their effects on host fitness have been studied and increasingly, we are gaining insight into the benefits and costs of their associations. Although some nutritional benefits attributed to their bacterial biosynthetic and metabolic capabilities are possible, experiments have focused mostly on their environmental effects. Those include detrimental consequences on growth and reproduction to the host (Koga et al. 2003; Sakurai et al. 2005). The reported benefits that facultative mutualists confer include protection against natural enemies (Ferrari et al. 2004; Guay et al. 2009; Oliver et al. 2005; Scarborough et al. 2005), heat damage (Chen et al. 2000; Montllor et al. 2002) or stress (Russell and Moran 2006). They are involved in host plant specialization and reproduction (Simon et al. 2003; Ferrari et al. 2004; Tsuchida et al. 2004), and even compensate the loss of the essential endosymbiont, as it was experimentally proven (Koga et al. 2003). Some facultative symbionts are reproductive manipulators, parasites that manipulate host reproduction to spread by increasing matrilineal transmission through reproductive incompatibility, son killing, feminization of genetic males and parthenogenesis. This is the case of *Wolbachia pipientis*, "*Ca*. Cardinium hertigii", *Arsenophus nasoniae*, *Spiroplasma*, some *Rickettsia* and ladybird male killers. Regarding the possible routes of transmission, it has been suggested that in some cases parasitic wasps can act as vectors of transmission (Vavre et al. 1999). Moreover, given the similarity between some symbionts found in aphids and ladybirds, such as *Rickettsia* (Werren et al. 1994), *Wolbachia* (Hurst et al. 1999b) and *Spiroplasma* (Hurst et al. 1999a), it has been postulated that predator–prey interactions also play a role in the transmission of these bacteria. Since many of these bacteria have an affinity for bacteria found in the intestines of these insects, has also suggested an oral route of transmission through food by plants whose surface has molasses, remains of other individuals or other infected phloem insects, something

possible in crowded colonies. All of these bacteria are accessory for their hosts since the infected insects can survive, and often even increase their fitness, in the absence of them. However that does not imply that the opposite is true. Thus, despite the fact that some of these S-symbionts having closely related free-living bacteria, as for example "*Ca*. Serratia symbiotica", they have never been found in the environment and are not cultivable, relying on the aphid and probably the P-symbiont *B. aphidicola* for its own survival.

A second step toward integration into a more close relationship is constituted by obligate mutualists, which become essential for the survival and reproduction of the insect. Their role becomes less environmentally dependent than in facultative symbionts and more nutritional and/or metabolic. Within the obligate symbionts, several stages of integration, revealed by their genome features can be distinguished. The most recent stages of this association are represented by clades of bacteria that have recently established such associations, such as SOPE and SZPE, the P-endosymbiont of the rice weevil *Sitophilus oryzae* and the maize weevil *Sitophilus zeamais*, respectively. These obligate mutualistic endosymbionts are closely related with the S-symbiont of tsetse flies, *S. glossinidius* (Dale and Welburn 2001; Heddi et al. 1998), but unlike it they cannot be cultured outside the host. SOPE and SZPE provide their hosts with amino acids and vitamins, having effects on fertility, development and the flying ability of adult insects (Heddi et al. 1999). The bacteria live inside bacteriocytes organized in an organ called bacteriome surrounding the midgut of the insect and near the female ovaries. With an estimated 3 Mb genome (Charles et al. 1997), within the range of many free-living bacteria, the association of this gamma-proteobacteria is not antique. Actually, some data indicate a recent endosymbiont replacement of an ancestral endosymbiont in the family Dryophtoridae to which the rice and maize weevils belong (Lefevre et al. 2004). Big amounts of transposable elements mainly IS elements have been identified in the SOPE and SZPE genomes (Gil et al. 2008; Plague et al. 2008). This abundance of repetitive DNA was unexpected in obligate mutualistic endosymbionts, because bacterial endosymbionts with a long-established obligatory relationship with their hosts show total absence of phages or transposable elements, whereas repetitive DNA is common in free-living bacteria, especially in recent specialized pathogens (e.g. the enteric bacteria *Shigella* and *Salmonella enterica* Typhi) (Jin et al. 2002; Wei et al. 2003), intracellular parasites (e.g. *W. pipientis* strains) (Klasson et al. 2008, 2009; Wu et al. 2004), or facultative insect symbionts (e.g. "*Ca*. Hamiltonella defensa", "*Ca*. Arsenophonus arthropodicus" and "*Ca*. Regiella insecticola") (Dale and Moran 2006; Degnan et al. 2009, 2010). The IS are able to move within genomes and also between genomes of different organisms by horizontal gene transfer. In free-living bacteria the IS transposition is tightly controlled, so that only a few copies of a limited number of categories appear in each genome. Their dramatic increase in intracellular bacteria must reflect the uncontrolled proliferation of elements that were already present at the beginning of symbiosis. This proliferation could be involved in the inactivation of non-essential genes and can also serve as a substrate for unequal recombination, thus promoting genome size reduction and genome rearrangements in early stages.

After the establishment of an obligate endosymbiont lifestyle, repetitive DNA tends to diminish until its total disappearance as observed in old endosymbionts, where the current lack of repetitive sequences, appears to be in the origin of their high genomic architecture stability levels (Silva et al. 2003). However, the early presence of such repetitive elements in the past might explain the genome reorganizations observer when compared to their free-living relatives. This is an indication that most of genomic modeling, including chromosomal rearrangements and the loss of many functionally dispensable genes, must take place at an early stage of the process of genomic adaptation to intracellular life (Dougherty and Plague 2008; Touchon and Rocha 2007). In addition to their genomic architecture stability, other genomic features of the long-established P-endosymbionts, many other characteristics indicate a convergent evolution among groups of bacteria non-related belonging to the Gammaproteobacteria and Bacteroidetes (López-Sánchez et al. 2009). These mature endosymbionts constitute the first and most studied ones so far (Buchner 1965), and have been considered the archetype of the mutualistic symbioses in arthropods. In general, these endosymbionts have highly compact genomes eight to ten times smaller than those of their free-living relatives. The reduced genome size of their genomes correlates quite well with the presence of a smaller number of genes compared to their free-living relatives. Additional characteristic genome features include almost total absence of recombination, increased rate of nucleotide substitution, high A + T content (although this no longer can be considered a general trait), accumulation of deleterious mutations by random genetic drift, loss of codon bias toward A or T, and accelerated sequence evolution (Andersson and Kurland 1998; Clark et al. 1999; Moya et al. 2002; Wernegreen 2005). The striking similarities in independently evolved obligate symbiont genomes indicate that common forces are at work. The aforementioned population dynamics of these microorganisms, including elevated genetic drift resulting from relaxed selection in populations with small effective size, frequent bottlenecks and horizontal transmission among hosts eliminated has as result the elevated fixation of mildly deleterious mutations, lower stability of proteins and inactivation and loss of non-essential genes. Nonetheless, variations according to the age of the association or the host lifestyle exist among these bacteria. Some adaptive changes include the plasmid-associated amplification of genes for biosynthesis of leucine and tryptophan (Latorre et al. 2005) in *B.aphidicola*, with different arrangements in divergent lineages, or the highly constitutive expression of the chaperone GroEL in *B.aphidicola* (Fares et al. 2002, 2004).

The fact that even long-established symbionts are still suffering a reductive process is illustrated by *B.aphidicola* where different strains from several aphid subfamilies show differences up to 200 Kb (Gil et al. 2002), and present pseudogenes and differential gene losses in the *B. aphidicola* genomes that have been sequenced (Perez-Brocal et al. 2006; Shigenobu et al. 2000; Tamas et al. 2002; van Ham et al. 2003). Those losses are randomly affecting different genes in each genome in a way that correlates with their hosts since the different strains of the bacteria diverged from their last common symbiont ancestor (LCSA).

In addition to a gradual reduction in the genome size, P-endosymbionts with an old association with their hosts have in general an increase in the A + T content compared with P-endosymbionts with a younger association and with S-symbionts, which in turn have intermediate values between the former and free-living bacteria. This enrichment in A + T has been related to the loss of DNA repair enzymes. However, several cases that do not follow this nucleotide composition rule, such as "*Ca*. Tremblaya princeps", the P-endosymbiont of the mealybug *Planococcus citri*, and "*Ca*. Hogkinia cicadicola", P-endosymbiont of the cicada *Dieroprocta semicincta* with a 57.1 and a 58.4% G + C content, respectively (Baumann et al. 2002; McCutcheon et al. 2009), which suggest that mutational pressure favoring A + T is not a critical step in endosymbiont genome evolution.

The evolution of the endosymbiotic integration through a random process of gradual pseudogenization and gene loss scattered throughout the genome (Gomez-Valero et al. 2004; Silva et al. 2001) might, theoretically, lead to the loss of all genes except those involved in the symbiotic relationship, as well as a reduced repertoire of genes necessary to maintain the essential functions. However, there are several cases described of extremely reduced genomes that have exceeded this threshold and have lost part of such essential functions. The most striking cases is that of "*Ca*. Carsonella ruddii", initially considered the P-endosymbiont of the psylid *Pachpsylla venusta*, with a 160 Kb genome, and only 182 predicted open reading frames, many of which overlapping (Nakabachi et al. 2006). The analysis of its coding capacities revealed that the extensive degradation of the genome is affecting vital and symbiotic functions (Tamames et al. 2007). "Thus, "*Ca*. Carsonella rudii" is not able to sustain its own essential life-related functions since most genes for DNA replication, transcription and translation are absent, as well as essential domains of many proteins due to a gene length shortening. In addition, "*Ca*. Carsonella ruddii" is not able to sustain the requirements of its host since the pathways for the synthesis of several essential amino acids are missing. Since it appears to be the sole symbiont in its host, one explanation is that somehow this insect can enhance the supply of nutrients provisioned by plants, as other sap-feeders do (Larson and Whitham 1991) in which case the former P-endosymbiont might become expendable and therefore nearing extinction. Alternatively it might be possible that some "*Ca*. Carsonella ruddii" genes have been transferred to the host nuclear DNA, as proved for present organelles. In this case "*Ca*. Carsonella ruddii" could be considered as a subcellular new entity between living cells and organelles (Tamames et al. 2007). The status as a cell or organelle and the fate of "*Ca*. Carsonella ruddii" represent an enigma that has yet to be clarified.

In other cases of extreme genomes, which are also reduced beyond the theoretical capacity of maintenance of vital and symbiotic functions, we can distinguish two scenarios offering more well-supported explanations. Both imply the eventual addition of a second bacterium in an existing symbiotic association between a bacterium and an animal host. Although at first this novel association can be facultative, the second bacterium can become essential for host fitness if it provides benefits to the organization. As a result, the evolutionary process of

genome shrinkage will affect both bacteria of the association, as new genes become redundant. However, which one of the two bacterial genomes loses them will be a matter of chance. Depending on which genome is affected by the loss of genes needed for the synthesis of essential molecules, either one bacterium can enter an extreme degenerative process, which may end with its extinction (replacement), and the retained bacteria will continue the degenerative process alone (Moya et al. 2009) or both of them will become indispensable to keep a healthy consortium (complementation). Replacement has already been reported, for example, in the Family Dryophthoridae, where a former endosymbiont "*Ca*. Nardonella" was replaced by the ancestor of the Sitophilus P-endosymbionts (Lefevre et al. 2004). However, there are many more described cases of consortia reported. One case of metabolic complementation involves *B. aphidicola* and "*Ca*. Serratia symbiotica" in the aphid *Cinara cedri*. "*Ca*. Serratia symbiotica" appears as a facultative symbiont in many aphid species. However, it is reported to have become an obligate symbiont in the cedar aphid (Lamelas et al. 2008), where it coexists with *B. aphidicola* BCc in the insect bacteriome. Perez-Brocal et al. (2006) proposed that "*Ca*. Serratia symbiotica" SCc might be replacing B *aphidicola* BCc because, unlike other sequenced *B.aphidicola* strains, it has partially lost its symbiotic role, as it cannot synthesize the essential amino acid tryptophan. However, using a metagenomic approach Gosalbes et al. 2008 discovered a plasmid in *B.aphidicola* BCc containing the *trpEG* genes, coding for anthranilate synthase, the first enzyme of the tryptophan biosynthesis pathway. The remaining genes for the pathway (*trpDCBA*) are located in the chromosome of "*Ca*. Serratia symbiotica". These data show that both endosymbionts are involved in the tryptophan biosynthesis that supplies both their host and themselves. *B.aphidicola* produces a metabolic intermediate that is then provided to "*Ca*. Serratia symbiotica" to synthesize the final product. Therefore, both bacteria coexist in intracellular obligatory mutualistic association with their host. The establishment of an endosymbiotic bacterium consortium can be in the origin of big evolutionary changes in host lifestyle. This could be the case of the following example. At least three different consortia involving the Bacteroidetes member "*Ca*. Sulcia muelleri" and three non-related co-primary symbionts have been characterized. The first one in the xylem-feeding sharpshooter *Homalodisca vitripennis*, with the Gammaproteobacterium "*Ca*. Baumannia cicadellinicola" (Wu et al. 2006), which is one with largest genome of the three co-resident bacteria (686 Kb) and with a 33% GC. The second one is found in the cicada *Diceroprocta semicincta*, with the Alphaproteobacterium "*Ca*. Hodgkinia cicadicola" (McCutcheon et al. 2009), the one with the most reduced genome size of any know bacterium (144 kb) but surprisingly high G + C content (58.4%). More recently the consortium formed in the spittlebug *Clastoptera arizonana* has been reported with the Betaproteobacterium "*Ca*. Zinderia insecticola" (McCutcheon and Moran, 2010) with a tiny genome of 208 kb and the most extreme nucleotide composition (13.5% G + C) reported to date The three strains of "*Ca*. Sulcia muelleri" differ in their genome size (from 245 to 276 kb), G + C content (21.1–22.6%) and gene content (e.g. lacks the *trp* operon in the spittlebug, which is

encoded by the two other strains). In all cases their whole genome analysis revealed that they have complementary sets of biosynthetic capabilities needed to provide to their host the nutrients that are lacking in the xylem sap. "*Ca*. Sulcia muelleri" encodes the enzymes involved in the biosynthesis of eight of the ten essential amino acids, whereas co-resident symbionts synthesize the remaining two. Interestingly, in the most recently described system, that of *C.arizonana*, "*Ca*. Sulcia muelleri" has completely lost the pathway for the biosynthesis of tryptophan and, therefore, retains the ability to make only seven of the ten essential amino acids, being the remaining three encoded by "*Ca*. Zinderia insecticola". "*Ca*. Baumannia cicadellinicola" contains a large number of pathways for biosynthesis of vitamins and cofactors, a role less evident in "*Ca*. Hodgkinia cicadicola", since it has lost all vitamin and cofactor biosynthetic abilities, implying that the cicada and its symbionts must access to external sources of these compounds, possibly from plant root xylem.

Another symbiotic consortium is that established between the sap-sucking mealybugs and the nested endosymbiotic system that comprises two bacteria: the P-endosymbiont "*Ca*. Tremblaya princeps", a Betaproteobacterium, which contains inside a Gammaproteobacterium (von Dohlen et al. 2001), considered as an S-symbiont based on its polyphyletic origin (Thao et al. 2002). The S-endosymbionts are distinct in each mealybug species and also differ from other insect-associated bacteria. "*Ca*. Tremblaya princeps" was the first endosymbiotic genome in which a high G + C content was found (57.1% for a fragment of 64 kb of its DNA) (Baumann et al. 2002). This is one of the very few described cases of a double endosymbiosis, [another one is the nested endosymbiosis of molgulid tunicates with the protist *Nephromyces* which is itself chronically infected with Gram-negative, intracellular bacteria, (Saffo, 1990)] and the first involving arthropods, although the symbiotic relationship between the two bacteria has not been elucidated (Kono et al. 2008).

In an example of convergent intracellular lifestyle, the genome reduction and the general (although not universal) trend toward lower GC content are also two characteristics of obligate intracellular pathogens, although the extremes reached by the insect endosymbionts have not been matched by any parasite. The most extreme cases are found within the Mollicutes. Thus, genera *Mycoplasma* (0.58–1.36 Mb, 23.8–40% GC), *Phytoplasma*. (0.60–0.88 Mb, 21.4–27.8% GC), *Ureaplasma*. (0.75–0.87 Mb, 25.5–25.8% GC) and *Mesoplasma* (0.79 Mb, 27.0% GC) account for the smallest genomes among parasitic bacteria. The intracellular lifestyle also imposed reduced genomes in other non-related bacteria, such as genera *Borrelia* (0.92–1.57 Mb, 27.5–29.8% GC), *Chlamydia* (1.00–1.08 Mb, 40.3–41.3% GC), *Chlamydophila* (1.14–1.23 Mb, 39.2–40.6% GC) or *Rickettsia* (1.10–1.58 Mb, 28.9–32.6% GC). This trend is evident even for the most extremely reduced genome described in archaea so far, that of *Nanoarchaeum equitans* (0.49 Mb, 31.6% GC), only known archaeal parasite, in this case of the Crenarcheote *Ignococcus hospitalis* (Waters et al. 2003). Therefore, there are universal principles that affect host-associated bacteria that transcends the character of the relationship with the host and that shape the genome of the bacterial partners, making our efforts ineffective to classify the whole diversity of the symbionts in a single and unambiguous way.

5 Concluding Remarks

The traditional textbook division of symbionts as parasites, commensals and mutualists no longer prevails as a set of discrete categories. Although intracellular obligate symbionts can usually be ascribed to either of them, virtually any criterion employed can bump into exceptions to the rules when it comes to the enormous quantity of newly identified and sequenced genomes of more labile symbionts, be they opportunistic pathogens or facultative mutualists. As shown in this review, many beneficial or detrimental effects can be defined only for a particular environment and under particular conditions, which are under constant transformation, imposing changing evolutionary pressures on the partners. We have shown how typical characteristics that traditionally have been ascribed to mutualists and parasites have, in fact examples from the each side that breach the norm, such as the mode of transmission, the mechanisms of invasion, the phylogeny. Also, transitions between mutualism and parasitism and vice versa are possible, even in evolutionary short periods of time. Even a priori considered essential long-established mutualists can become non-essential, losing the mutualistic role and therefore opening the door to extreme degradation and extinction and/or replacement. Due to the novel methodologies the study of symbiotic relationships is entering a new age beyond the study of relatively simple systems composed by two species. As more multiple interactions involving several bacteria, which interact the ones with the others in addition to the host, are reported the more this knowledge may help us in understanding relationships in more complex microbial communities, such as the human, animal or environmental microbiomes.

Acknowledgments Financial support was provided by grants BFU2009-12895-C02-01 (Ministerio de Ciencia e Innovación, Spain) to A. Latorre, and SAF2009-13302-C02-01 (Ministerio de Ciencia e Innovación, Spain) and PROMETEO/2009/092 (Conselleria d'Educació, Generalitat Valenciana, Spain) to A. Moya

References

Agnew P, Koella JC (1997) Virulence, parasite mode of transmission, and host fluctuating asymmetry. Proc Biol Sci 264:9–15

Andersson SG, Kurland CG (1998) Reductive evolution of resident genomes. Trends Microbiol 6:263–268

Anselme C, Vallier A, Balmand S, Fauvarque MO, Heddi A (2006) Host PGRP gene expression and bacterial release in endosymbiosis of the weevil *Sitophilus zeamais*. Appl Environ Microbiol 72:6766–6772

Baumann L, Thao ML, Hess JM, Johnson MW, Baumann P (2002) The genetic properties of the primary endosymbionts of mealybugs differ from those of other endosymbionts of plant sapsucking insects. Appl Environ Microbiol 68:3198–3205

Buchner P (1965) Endosymbiosis of animals with plant microorganisms. Interscience Publishers, New York

Bull JJ, Molineux IJ, Rice WR (1991) Selection of benevolence in a host–parasite system. Evolution 45:875–882

Champion de Crespigny FE, Wedell N (2006) *Wolbachia* infection reduces sperm competitive ability in an insect. P Roy Soc B-Biol Sci 273:1455–1458

Charles H, Heddi A, Guillaud J, Nardon C, Nardon P (1997) A molecular aspect of symbiotic interactions between the weevil *Sitophilus oryzae* and its endosymbiotic bacteria: over-expression of a chaperonin. Biochem Biophys Res Commun 239:769–774

Chen D-Q, Montllor CB, Purcell AH (2000) Fitness effects of two facultative endosymbiotic bacteria on the pea aphid, *Acyrthosiphon pisum*, and the blue alfalfa aphid *A. kondoi*. Entomol Exp Appl 95:315–323

Clark MA, Moran NA, Baumann P (1999) Sequence evolution in bacterial endosymbionts having extreme base compositions. Mol Biol Evol 16:1586–1598

Cushman JH, Beattie AJ (1991) Mutualisms: assessing the benefits to hosts and visitors. Trends Ecol Evol 6:193–195

Dale C, Maudlin I (1999) Sodalis gen. nov. and *Sodalis glossinidius* sp. nov., a microaerophilic secondary endosymbiont of the tsetse fly *Glossina morsitans morsitans*. Int J Syst Bacteriol 49:267–275

Dale C, Moran NA (2006) Molecular interactions between bacterial symbionts and their hosts. Cell 126:453–465

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

Dale C, Young SA, Haydon DT, Welburn SC (2001) The insect endosymbiont *Sodalis glossinidius* utilizes a type III secretion system for cell invasion. In: Proceedings of National Academy of Science, USA 98:1883–1888

Dale C, Plague GR, Wang B, Ochman H, Moran NA (2002) Type III secretion systems and the evolution of mutualistic endosymbiosis. In: Proceedings of National Academy of Science 99:12397–12402

Darby AC, Cho NH, Fuxellus HH, Westberg J, Andersson SGE (2007) Intracellular pathogens go extreme: genome evolution in the Rickettsiales. Trends Genet 23:511–520

Davis MM, Bjorkman PJ (1988) T-cell antigen receptor genes and T-cell recognition. Nature 334:395–401

Day T (2001) Parasite transmission modes and the evolution of virulence. Evolution 55:2389–2400

Degnan H, Lazarus AB, Wernegreen JJ (2005) Genome sequence of *Blochmannia pennsylvanicus* indicates parallel evolutionary trends among bacterial mutualists of insects. Ge-nome Res 15:1023–1033

Degnan PH, Leonardo TE, Cass BN, Hurwitz B, Stern D, Gibbs RA, Richards S, Moran NA (2010) Dynamics of genome evolution in facultative symbionts of aphids. Environ Microbiol 12:2060–2069

Degnan PH, Yu Y, Sisneros N, Wing RA, Moran NA (2009) *Hamiltonella defensa*, genome evolution of protective bacterial endosymbiont from pathogenic ancestors. Proc Natl Acad Sci USA 106:9063–9068

Dionne MS, Schneider DS (2008) Models of infectious diseases in the fruit fly *Drosophila* melanogaster. Dis Model Mech 1:43–49

Dougherty KM, Plague GR (2008) Transposable element loads in a bacterial symbiont of weevils are extremely variable. Appl Environ Microbiol 74:7832–7834

Ecker DJ, Sampath R, Willett P, Wyatt JR, Samant V, Massire C, Hall TH, Hari K, McNeil JA, Büchen-Osmond C, Budowle B (2005) The microbial rosetta stone database: a compilation of global and emerging infectious microorganisms and bioterrorist threat agents. BMC Microbiol 5:19

Edwards RA, Olsen GJ, Maloy SR (2002) Comparative genomics of closely related Salmonellae. Trends Microbiol 10:94–99

Everett KDE, Thao M, Horn M, Dyszynski GE, Baumann P (2005) Novel chlamydiae in whiteflies and scale insects: endosymbionts '*Candidatus* Fritschea bemisiae' strain Falk and '*Candidatus* Fritschea eriococci' strain Elm. Int J Syst Evol Microbiol 55:1581–1587

Ewald PW (1995) The evolution of virulence: a unifying link between parasitology and ecology. J Parasitol 81:659–669
Falkow S (1997) What is a pathogen? ASM News 7:359–365
Fares MA, Ruiz-González MX, Moya A, Elena SF, Barrio E (2002) Endosymbiotic bacteria: GroEL buffers against deleterious mutations. Nature 417:398
Fares MA, Moya A, Barrio E (2004) GroEL and the maintenance of bacterial endosymbiosis. Trends Genet 20:413–416
Feldhaar H, Gross R (2008) Immune reactions of insects on bacterial pathogens and mutualists. Microb Infect 10:1082–1088
Ferdy JB, Godelle B (2005) Diversification of transmission modes and the evolution of the mutualism. Am Nat 166:613–627
Ferrari J, Darby AC, Daniell HCJG, Douglas AE (2004) Linking the bacterial community in pea aphids with host-plant use and natural enemy resistance. Ecol Entomol 29:60–65
Ferrière R, Gauduchon M, Bronstein JL (2007) Evolution and persistence of obligate mutualists and exploiters: competition for partners and evolutionary immunization. Ecol Lett 10:115–126
Fleury F, Vavre F, Ris N, Fouillet P, Boulétreau M (2000) Physiological cost induced by the maternally-transmitted endosymbiont *Wolbachia* in *Drosophilla* parasitoid *Leptopilina heterotoma*. Parasitology 121:493–500
Fraser CM, Gocayne JD, White O, Adams MD, Clayton RA, Fleischmann RD, Bult CJ, Kerlavage AR, Sutton G, Kelley JM, Fritchman RD, Weidman JF, Small KV, Sandusky M, Fuhrmann J, Nguyen D, Utterback TR, Saudek DM, Phillips CA, Merrick JM, Tomb JF, Dougherty BA, Bott KF, Hu PC, Lucier TS, Peterson SN, Smith HO, Hutchison CA, Venter JC (1995) The minimal gene complement of *Mycoplasma genitalium*. Science 270:397–403
Fukatsu T, Nikoh N, Kawai R, Koga R (2000) The secondary endosymbiotic bacterium of the pea aphid *Acyrthosiphon pisum* (Insecta: Homoptera). Appl Environ Microbiol 66:2748–2758
Fytrou A, Schofield PG, Kraaijeveld AR, Hubbard SF (2006) *Wolbachia* infection sup-presses both host defence and parasitoid counter-defence. P Roy Soc B-Biol Sci 273:791–796
Gerardo NM, Altincicek B, Anselme C, Atamian H, Barribeau SM, de Vos M, Duncan EJ, Evans JD, Gabaldón T, Ghanim M, Heddi A, Kaloshian I, Latorre A, Moya A, Nakabachi A, Parker BJ, Pérez-Brocal V, Pignatelli M, Rahbé Y, Ramsey JS, Spragg CJ, Tamames J, Tamarit D, Tamborindeguy C, Vincent-Monegat C, Vilcinskas A (2010) Immunity and other defenses in pea aphids, *Acyrthosiphon pisum*. Genome Biol. doi: 10.1186/gb-2010-11-2-r21
Gil R, Silva FJ, Zientz E, Delmotte F, Gonzalez-Candelas F, Latorre A, Rausell C, Kamerbeek J, Gadau J, Holldobler B, van Ham RCHJ, Gross R, Moya A (2003) The genome sequence of *Blochmannia floridanus*: comparative analysis of reduced genomes. In: Proceedings of National Academy of Science, USA 100:9388–9393
Gil R, Latorre A, Moya A (2004) Bacterial endosymbionts of insects: insights from comparative genomics. Environ Microbiol 6:1109–1122
Gil R, Belda E, Gosalbes MJ, Delaye L, Vallier A, Vincent-Monegat C, Heddi A, Silva FJ, 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 (2010) Evolution of prokaryote-animal symbiosis from a genomics perspective. Microbiol Monographs 19:207–233
Gil-Turnes MS, Hay ME, Fenical W (1989) Symbiotic marine bacteria chemically defend crustacean embryos from a pathogenic fungus. Science 246:116–118
Gil-Turnes MS, Fenical W (1992) Embryos of *Homarus americanus* are protected by epibiotic bacteria. Biol Bull 182:105–108
Goebel W, Gross R (2001) Intracellular survival strategies of mutualistic and parasitic prokaryotes. Trends Microbiol 9:267–273
Gomez-Valero L, Latorre A, Silva FJ (2004) The evolutionary fate of nonfunctional DNA in the bacterial endosymbiont *Buchnera aphidicola*. Mol Biol Evol 21:2172–2181
Gosalbes MJ, Lamelas A, Moya A, Latorre A (2008) The striking case of tryptophan provision in the cedar aphid *Cinara cedri*. J Bacteriol 190:6026–6029

Gosalbes MJ, Latorre A, Lamelas A, Moya A (2010) Genomics of intracellular symbionts in insects. Int J Med Microbiol 300:271–278

Gottlieb Y, Ghanim M, Gueguen G, Kontsedalov S, Vavre F, Fleury F, Zchori-Fein E (2008) Inherited intracellular ecosystem: symbiotic bacteria share bacteriocytes in whiteflies. FASEB J 22:2591–2599

Govind S (2008) Innate immunity in *Drosophila*: pathogens and pathways. Insect Sci 8(15):29–43

Guay J-F, Boudreault S, Michaud D, Cloutier C (2009) Impact of environmental stress on aphid clonal resistance to parasitoids: role of *Hamiltonella defensa* bacterial symbiosis in association with a new facultative symbiont of the pea aphid. J Insect Physiol 55:919–926

Hansen AK, Jeong G, Paine TD, Stouthamer R (2007) Frequency of secondary symbiont infection in an invasive psyllid relates to parasitism pressure on a geographic scale in California. Appl Environ Microbiol 73:7531–7535

Hao Z, Kasumba I, Lehane MJ, Gibson WC, Kwon J, Aksoy S (2001) Tsetse immune responses and trypanosome transmission: implications for the development of tsetse-based strategies to reduce trypanosomiasis. In: Proceedings of National Acadmy of Science, USA 98:12648–12653

Haynes S, Darby AC, Daniell TJ, Webster G, van Veen FJF, Godfray HCJ, Prosser JI, Douglas AE (2003) Diversity of bacteria associated with natural aphid populations. Appl Envir Microbiol 69:7216–7223

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 AM, Khatchadourian C, Charles H, Nardon P (1999) Four intracellular genomes direct weevil biology: nuclear, mitochondrial, principal endosymbiont, and *Wolbachia*. In: Proceedings of National Academy of Science 96:6814–6819

Hosokawa T, Koga R, Kikuchi Y, Meng XY, Fukatsu T (2010) *Wolbachia* as a bacteriocyte-associated nutritional mutualist. In: Proceedings of National Academy of Science 107:769–774

Hurst GD, Graf von der Schulenburg JH, Majerus TM, Bertrand D, Zakharov IA, Baungaard J, Völkl W, Stouthamer R, Majerus ME (1999a) Invasion of one insect species, *Adalia bipunctata*, by two different male-killing bacteria. Insect Mol Biol 8:133–139

Hurst GDD, Jiggins FM, von der Schulenberg JHG, Bertrand D, West SA, Goriacheva II, Zakharov MEN, Werren JH, Stouthamer R, Majerus EN (1999b) Male-killing *Wolbachia* in *Trichogramma* wasps. P Roy Soc B Biol Sci 266:735–740

Jiggins FM, Hurst GDD, Jiggins CD, von der Schulenburg JHG, Majerus MEN (2000) The butterfly *Danaus chrysippus* is infected by a male-killing *Spiroplasma* bacterium. Parasitology 120:439–446

Jin Q, Yuan Z, Xu J, Wang Y, Shen Y, Lu W, Wang J, Liu H, Yang J, Yang F, Zhang X, Zhang J, Yang G, Wu H, Qu D, Dong J, Sun L, Xue Y, Zhao A, Gao Y, Zhu J, Kan B, Ding K, Chen S, Cheng H, Yao Z, He B, Chen R, Ma D, Qiang B, Wen Y, Hou Y, Yu J (2002) Genome sequence of *Shigella flexneri* 2a: insights into pathogenicity through comparison with genomes of *Escherichia coli* K12 and O157. Nucleic Acids Res 30:4432–4441

Klasson L, Walker T, Sebaihia M, Sanders MJ, Quail MA, Lord A, Sanders S, Earl J, O'Neill SL, Thomson N, Sinkins SP, 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, Naslund K, Lutnaes Y, Darby AC, Veneti Z, Chen L, Braig HR, Garrett R, Bourtzis K, Andersson SG (2009) The mosaic genome structure of the *Wolbachia* wRi strain infecting *Drosophila simulans*. In: Proceedings of National Academy of Science 106:5725–5730

Koga R, Tsuchida T, Fukatsu T (2003) Changing partners in an obligate symbiosis: a facultative endosymbiont can compensate for loss of the essential endosymbiont *Buchnera* in an aphid. Proc Biol Sci 270:2543–2550

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

Kurtz J, Armitage SAO (2006) Alternative adaptive immunity in invertebrates. Trends Immunol 27:493–496

Lamelas A, Pérez-Brocal V, Gómez-Valero L, Gosalbes MJ, 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

Langworthy NG, Renz A, Mackenstedt U, Henkle-Duhrsen K, De Bronsvoort MB, Tanya VN, Donnelly MJ, Trees AJ (2000) Macrofilaricidal activity of tetracycline against the filarial nematode *Onchocerca ochengi*: elimination of *Wolbachia* precedes worm death and suggests a dependent relationship. Proc R Soc Lond B Biol Sci 267:1063–1069

Larson KC, Whitham TG (1991) Manipulation of food resources by a gall-forming aphid: the physiology of sink-source interactions. Oecologia 88:15–21

Latorre A, Gil R, Silva FJ, Moya A (2005) Chromosomal stasis versus plasmid plasticity in aphid endosymbiont *Buchnera aphidicola*. Heredity 95:339–347

Lebecque SG, Bearhart PJ (1990) Boundaries of somatic mutation in rearranged immunoglobulin genes: 5b boundary is near the promoter and 3b boundary is 1 Kb from V(D)J gene. J Exp Med 172:1717–1727

Lefevre 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

Leung TLF, Poulin R (2008) Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. Vie Milieu 58:107–115

López-Sánchez MJ, 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:e1000721

Male D (2004) Immunology. Elsevier, London

Margulis L (1981) Symbiosis in cell evolution, 1st edn. Freeman, New York

Margulis L (1993) Symbiosis in Cell Evolution, 2nd edn. Freeman, New York

McCutcheon JP, Moran NA (2007) Parallel genomic evolution and metabolic interdependence in an ancient symbiosis. In: Proceedings of National Academy of Science 104:19392–19397

McCutcheon JP, Moran NA (2010) Functional convergence in reduced genomes of bacterial symbionts spanning 200 My of evolution. Genome Biol Evol 2:708–718

McCutcheon JP, McDonald BR, Moran NA (2009) Origin of an alternative genetic code in the extremely small and GC-rich genome of a bacterial symbiont. PLoS Genet 5:e1000565

Meyers RA (2007) Immunology: from cell biology to disease. Wiley, Weinheim

Miller MR, White A, Boots M (2006) The evolution of parasites in response to tolerance in their host: the good, the bad, and apparent commensalism. Evolution 60:945–956

Mitsuhashi W, Saiki T, Wei W, Kawakita H, Sato M (2002) Two novel strains of Wolbachia coexist in both species of mulberry leafhoppers, *Hishimonoides sellatiformis* and *Hishimonus sell*atus which are vectors of mulberry dwarf phytoplasma. Insect Mol Biol 11:577–584

Montllor CB, Maxmen A, Purcell AH (2002) Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. Ecol Entomol 27:189–195

Moran NA (1996) Accelerated evolution and Muller's rachet in endosymbiotic bacteria. In: Proceedings of National Academy of Science USA 93:2873–2878

Moran NA (2007) Symbiosis as an adaptive process and source of phenotypic complexity. In: Proceedings of National Academy of Science 104:8627–8633

Moran NA, Wernegreen JJ (2000) Lifestyle evolution in symbiotic bacteria: insights from genomics. Trends Ecol Evol 15:321–326

Moran NA, Russell JA, Koga R, Fukatsu T (2005a) Evolutionary relationships of three new species of Enterobacteriaceae living as symbionts of aphids and other insects. Appl Environ Microbiol 71:3302–3310

Moran NA, Tran P, Gerardo NM (2005b) Symbiosis and insect diversification: an ancient symbiont of sapfeeding insects from the bacterial phylum Bacteroidetes. App Environ Microbiol 71:8802–8810

Moran NA, McCutcheon JP, Nakabachi A (2008) Genomics and evolution of heritable bacterial symbionts. Annu Rev Genet 42:165–190

Moya A, Latorre A, Sabater-Munoz B, Silva FJ (2002) Comparative molecular evolution of primary (*Buchnera*) and secondary symbionts of aphids based on two protein-coding genes. J Mol Evol 55:127–137

Moya A, Gil R, Latorre A (2009) The evolutionary history of symbiotic associations among bacteria and their animal hosts: a model. Clin Microbiol Infect 15:11–13

Müller WEG, Müller I (2003) Analysis of the sponge (Porifera) gene repertoire: implication for the evolution of the Metazoan body plan. In: Müller WEG (ed) Marine Molecular Biotechnology. Springer-Verlag, Berlin

Nakabachi A, Shigenobu S, Sakazume N, Shiraki T, Hayashizaki Y, Carninci P, Ishikawa H, Kudo T, Fukatsu T (2005) Transcriptome analysis of the aphid bacteriocyte, the symbiotic host cell that harbors an endocellular mutualistic bacterium, *Buchnera*. In: Proceedings of National Academy of Science 102:5477–5482

Nakabachi A, Yamashita A, Toh H, Ishikawa H, Dunbar HE, Moran NA, Hattori M (2006) The 160-kilobase genome of the bacterial endosymbiont Carsonella. Science 314:267

Nováková E, Hypsa V (2007) A new *Sodalis* lineage from bloodsucking fly *Craterina melbae* (Diptera, Hippoboscoidea) originated independently of the tsetse flies symbiont *Sodalis glossinidius*. FEMS Microbiol Lett 269:131–135

Oliver KM, Russell JA, Moran NA, Hunter MS (2003) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proc Natl Acad Sci USA 100:1803–1807

Oliver KM, Moran NA, Hunter MS (2005) Variation in resistance to parasitism in aphids is due to symbionts not host genotype. In: Proceedings of National Academy of Science 102:12795–12800

Oliver KM, Moran NA, Hunter MS (2006) Costs and benefits of a superinfection of facultative symbionts in aphids. P Roy Soc B Biol Sci 273:1273–1280

Oliver KM, Degnan PH, Hunter MS, Moran NA (2009) Bacteriophages encode factors required for protection in a symbiotic mutualism. Science 325:992–994

O'Neill SL, Giordano R, Colbert AME, Karr TL, Robertson HM (1992) 16S ribosomal RNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. In: Proceedings of National Academy of Science 89:2699–2702

Perez-Brocal V, Gil R, Ramos S, Lamelas A, Postigo M, Michelena JM, Silva FJ, Moya A, Latorre A (2006) A small microbial genome: the end of a long symbiotic relationship? Science 314:312–313

Perrot-Minnot MJ, Cheval B, Migeon A, Navajas M (2002) Contrasting effects of Wolbachia on cytoplasmic incompatibility and fecundity in the haplodiploid mite *Tetranychus urticae*. J Evol Biol 15:808–817

Pier GB, Lyczak JB, Wetzler LM (2004) Immunology, infection, and immunity. ASM Press, Washington DC

Plague GR, Dunbar HE, Tran PL, Moran NA (2008) Extensive proliferation of transposable elements in heritable bacterial symbionts. J Bacteriol 190:777–779

Poulsen M, Cafaro M, Boosma JJ, Currie CR (2005) Specificity of the mutualistic associ-ation between actinomycete bacteria and two sympatric species of Acromyrmex leaf-cutting ants. Mol Ecol 14:3597–3604

Rokita E, Makristathis A, Presterl E, Rotter ML, Hirschl AM (1998) Helicobacter pylori urease significantly reduces opsonization by human complement. J Infect Dis 178:1521–1525

Russell JA, Moran NA (2005) Horizontal transfer of bacterial symbionts: heritability and fitness effects in a novel aphid host. Appl Environ Microbiol 71:7987–7994

Russell JA, Moran NA (2006) Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. Proc Biol Sci 273:603–610

Russell JA, Latorre A, Sabater-Munoz B, Moya A, Moran NA (2003) Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea. Mol Ecol 12:1061–1075

Saffo MB (1990) Symbiosis within a symbiosis: Intracellular bacteria within the endosymbiotic protist *Nephromyces*. Marine Biol 107:291–296

Sakurai M, Koga R, Tsuchida T, Meng X-Y, Fukatsu T (2005) *Rickettsia* symbiont in the pea aphid Acyrthosiphon pisum: novel cellular tropism, effect on host fitness, and interaction with the essential symbiont *Buchnera*. Appl Environ Microbiol 71:4069–4075

Sandström JP, Russell JA, White JP, Moran NA (2001) Independent origins and horizontal transfer of bacterial symbionts of aphids. Mol Ecol 10:217–228

Sassera D, Beninati T, Bandi C, Bouman EAP, Sacchi L, Fabby 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

Scarborough CL, Ferrari J, Godfray HCJ (2005) Bacterial endosymbiont increases aphid inclusive fitness after pathogen attack. Science 310:1781

Schulenburg H, Boehnisch C, Michiels NK (2007) How do invertebrates generate a highly specific innate immune response? Mol Immunol 44:3338–3344

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

Silva FJ, Latorre A, Moya A (2001) Genome size reduction through multiple events of gene disintegration in *Buchnera* APS. Trends Genet 17:615–618

Silva FJ, Latorre A, Moya A (2003) Why are the genomes of endosymbiotic bacteria so stable? Trends Genet 19:176–180

Silverman DJ, Bound SB (1984) Infection of human vascular endothelial cells by *Rickettsia rickettsii*. J Infect Dis 149:201–206

Simon JC, Carre S, Boutin M, Prunier-Leterme N, Sabater-Mun B, Latorre A, Bournoville R (2003) Host-based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts. Proc Biol Sci 270:1703–1712

Steinert M, Hentschel U, Hacker J (2000) Symbiosis and pathogenesis: evolution of the microbe-host interaction. Naturwissenschaften 87:1–11

Stewart AD, Logsdon JM Jr, Kelley SE (2005) An empirical study of the evolution of virulence under both horizontal and vertical transmission. Evolution 59:730–739

Stouthamer R, Breeuwer JAJ, Hurst GDD (1999) *Wolbachia pipientis*: Microbial manipulator of arthropod reproduction. Annu Rev Microbiol 53:71–102

Strand MR (2008) The insect cellular immune response. Insect Sci 15:1–14

Sullivan JT, Trzebiatowski JR, Cruickshank RW, Gouzy J, Brown SD, Elliot RM, Fleet wood DJ, McCallum NG, Rossbach U, Stuart GS, Weaver JE, Webby RJ, De Bruijn FJ, Ronson CW (2002) Comparative sequence analysis of the symbiosis island of *Mesorhizobium loti* strain R7A. J Bacteriol 184:3086–3095

Tamames J, Gil R, Latorre A, Pereto J, Silva FJ, Moya A (2007) The frontier between cell and organelle: genome analysis of Candidatus Carsonella ruddii. BMC Evol Biol 7:181

Tamas I, Klasson L, Canback B, Naslund AK, Eriksson AS, Wernegreen JJ, Sandstrom JP, Moran NA, Andersson SG (2002) 50 million years of genomic stasis in endosymbiotic bacteria. Science 296:2376–2379

Thao ML, Gullan PJ, Baumann P (2002) Secondary (gamma-Proteobacteria) endosymbionts infect the primary (beta-Proteobacteria) endosymbionts of mealybugs multiple times and coevolve with their hosts. Appl Environ Microbiol 68:3190–3197

Toft C, Andersson SGE (2010) Evolutionary microbial genomics: insights into bacterial host adaptation. Nature Rev Genet 11:465–475

Toh H, Weiss BL, Perkin SA, 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

Tonegawa S (1983) Somatic generation of antibody diversity. Nature 302:575–581

Touchon M, Rocha EP (2007) Causes of insertion sequences abundance in prokaryotic genomes. Mol Biol Evol 24:969–981

Tsuchida TR, Koga R, Fukatsu T (2004) Host plant specialization governed by facultative symbiont. Science 303:1989

van Ham RC, Kamerbeek J, Palacios C, Rausell C, Abascal F, Bastolla U, Fernandez JM, Jimenez L, Postigo M, Silva FJ, Tamames J, Viguera E, Latorre A, Valencia A, Moran F, Moya A (2003) Reductive genome evolution in *Buchnera aphidicola*. In: Proceedings of National Academy of Science 100:581–586

Vavre F, Fleury F, Lepetit D, Fouillet P, Bouletreau M (1999) Phylogenetic evidence for horizontal transmission of *Wolbachia* in host-parasitoid associations. Mol Biol Evol 16:1711–1723

von Dohlen CD, Kohler S, Alsop ST, McManus WR (2001) Mealybug beta-proteobacterial endosymbionts contain gamma-proteobacterial symbionts. Nature 412:433–436

Waters E, Hohn MJ, Ahel I, Graham DE, Adams MD, Barnstead M, Beeson KY, Bibbs L, Bolanos R, Keller M, Kretz K, Lin X, Mathur E, Ni J, Podar M, Richardson T, Sutton GG, Simon M, Soll D, Stetter KO, Short JM, Noordewier M (2003) The genome of *Nanoarchaeum equitans*: insights into early archaeal evolution and derived parasitism. In: Proceedings of National Academy of Science 100:12984–12988

Weeks AR, Turelli M, Harcombe WR, Reynolds KT, Hoffmann AA (2007) From parasite to mutualist: Rapid evolution of *Wolbachia* in natural populations of *Drosophila*. PLoS Biol 5:e114

Wei J, Goldberg MB, Burland V, Venkatesan MM, Deng W, Fournier G, Mayhew GF, Plunkett G 3rd, Rose DJ, Darling A, Mau B, Perna NT, Payne SM, Runyen-Janecky LJ, Zhou S, Schwartz DC, Blattner FR (2003) Complete genome sequence and comparative genomics of *Shigella flexneri* serotype 2a strain 2457T. Infect Immun 71:2775–2786

Wernegreen JJ (2005) For better or worse: genomic consequences of intracellular mutualism and parasitism. Curr Opin Genet Dev 15:572–583

Werren JH, Hurst GDD, Zhang W, Breeuwer JAJ, Stouthamer R, Majerus MEN (1994) Rickettsial relative associated with male-killing in the ladybird beetle (*Adalia bipunctata*). J Bacteriol 176:388–394

Werren JH, Baldo L, Clark ME (2008) *Wolbachia*: master manipulators of invertebrate biology. Nat Rev Microbiol 6:741–751

Williamson DL, Poulson DF (1979) Sex ratio organisms (Spiroplasmas) of *Drosophila*. In: Whitcomb RF, Tully JG (eds) The Mycoplasmas. Academic Press, New York

Wu M, Sun LV, Vamathevan J, Riegler M, Deboy R, Brownlie JC, McGraw EA, Martin W, Esser C, Ahmadinejad N, Wiegand C, Madupu R, Beanan MJ, Brinkac LM, Daugherty SC, Durkin AS, Kolonay JF, Nelson WC, Mohamoud Y, Lee P, Berry K, Young MB, Utterback T, Weidman J, Nierman WC, Paulsen IT, Nelson KE, Tettelin H, O'Neill SL, Eisen JA (2004) Phylogenomics of the reproductive parasite *Wolbachia pipientis* wMel: a streamlined genome overrun by mobile genetic elements. PLoS Biol 2:E69

Wu D, Daugherty SC, Van Aken SE, Pai GH, Watkins KL, Khouri H, Tallon LJ, Zaborsky JM, Dunbar HE, Tran PL, Moran NA, Eisen JA (2006) Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. PLoS Biol 4:e188

Zchori-Fein E, Perlman SJ (2004) Distribution of the bacterial symbiont *Cardinium* in arthropods. Mol Ecol 13:2009–2016

Zeh JA, Zeh DW (2006) Male-killing *Wolbachia* in a live-bearing arthropod: brood abortion as a constraint on the spread of a selfish microbe. J Invertebr Pathol 92:33–38

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