Genomics and Evolution of Heritable Bacterial Symbionts

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Abstract

Insect heritable symbionts have proven to be ubiquitous, based on molecular screening of various insect lineages. Recently, molecular and experimental approaches have yielded an immensely richer understanding of their diverse biological roles, resulting in a burgeoning research literature. Increasingly, commonalities and intermediates are being discovered between categories of symbionts once considered distinct: obligate mutualists that provision nutrients, facultative mutualists that provide protection against enemies or stress, and symbionts such as *Wolbachia* that manipulate reproductive systems. Among the most far-reaching impacts of widespread heritable symbiosis is that it may promote speciation by increasing reproductive and ecological isolation of host populations, and it effectively provides a means for transfer of genetic information among host lineages. In addition, insect symbionts provide some of the extremes of cellular genomes, including the smallest and the fastest evolving, raising new questions about the limits of evolution of life.

INTRODUCTION

Bacteriome/ bacteriocyte: a host organ/cell specialized for housing bacterial symbionts, which usually are restricted to the cytoplasm

Whereas researchers tend to pay more attention to pathogenic bacteria, chronic bacterial infections that inflict no evident harm on their hosts are everywhere in eukaryotes. In invertebrate animals, such "symbiotic" bacteria are often inherited, usually maternally. While the prevalence of symbiosis has long been recognized on the basis of observations from microscopy (e.g., 16), most aspects of symbiont origins and functions were mysterious before the age of molecular techniques, as the uncultivability of symbionts hindered conventional microbial experiments. During the past 20 years, molecular studies on symbiont origins, evolution, and functions have dramatically increased our knowledge and appreciation of the importance of symbionts in the evolution of their host animals, plants, and other microorganisms. They have diverse ecological and evolutionary effects on hosts, influencing aspects of ecological interactions from nutrition to defense and affecting reproductive systems, with consequences for population structure, reproductive isolation, and speciation. Symbioses of insects and other arthropods have received the most study and are the focus of this review. However, inherited bacterial symbionts, with features paralleling those of insect symbionts, are known from many other animal phyla, including nematodes (140), sponges (130), annelids (59), bryozoans (133), and molluscs (79, 98).

Genome-based studies of insect symbioses have produced a large and rapidly expanding literature. In addition to providing new insights into the origins and evolution of symbionts and their many biological roles in hosts, this work has yielded remarkable and unexpected discoveries that challenge concepts of the limits of genome and cell evolution. Insect symbionts comprise some of the extremes of known biological diversity: Their genomes include the smallest known for any cellular organisms, completely lacking in mobile elements or bacteriophage; the most biased in base composition; the most stable in genome architecture; and the fastest in rate of gene sequence evolution (100, 134, 139). Others are rife with insertion sequences and genomic islands representing lysogenic or inactivated phage (e.g., 115, 143, 162).

This literature on molecular studies of heritable symbionts in insects is fragmented, reflecting the fact that, initially, symbiont types (i.e., parasites, commensalists, mutualists, etc.) were discovered through different routes and appeared dissimilar. However, recent results show that no firm boundaries separate categories of symbionts. In this review, we summarize findings on the broad spectrum of heritable insect symbionts, covering both their roles in hosts and their patterns of evolution.

Categories of Heritable Symbionts

Known heritable symbionts of insects are obligately symbiotic: So far as is known, they lack a replicative phase or dormant phase outside hosts. But they vary as to whether they are obligate from the host perspective, that is, whether they are required for successful host development and reproduction. Although intermediates and transitions are frequent, it is useful to recognize three categories of heritable symbiosis (**Figure 1**) (See sidebar: Symbiont Nomenclature).

The first category consists of obligate mutualists required to support normal host development. These symbionts, also called primary symbionts or P-symbionts, are typically restricted to a specialized organ, called a bacteriome, which consists of a set of distinctive host cells, called bacteriocytes. Depending on the host group, the bacteriome may consist of fat body cells, gut wall cells, or highly specialized cells that are developmentally determined in embryos (e.g., 13, 16). Bacteriome-associated symbionts, exemplified by *Buchnera aphidicola* in aphids, can be thought of as domesticated by hosts: They cannot invade naïve hosts and have evolved to be dependent on host-based mechanisms for transmission. Some obligate mutualists rival cell organelles in their extent of intimate association on hosts, as in the case of the extreme small genome symbiont, *Carsonella ruddii*, living in psyllids (100). Because bacteriome-associated symbionts often occupy conspicuous host organs, they were the focus of most studies of animal symbionts prior to the availability of molecular methods (e.g., 16, 43).

In contrast to obligate, bacteriome-associated symbionts, so-called facultative (or secondary or S-) symbionts are erratically distributed and are not required for host reproduction (62, 96, 145). Facultative symbionts resemble invasive pathogens in that they may invade various cell types, including reproductive organs, and may reside extracellularly in the body cavity (hemolymph) (38, 51). In many cases, facultative symbionts experimentally introduced to previously uninfected hosts establish stable, maternally inherited infection (18, 116), indicating that the persistence of the symbiosis is largely achieved through symbiont capabilities rather than host adaptations for maintaining symbiosis. In insects with bacteriomes, facultative symbionts may invade bacteriocytes where they coreside with, or even exclude, obligate symbionts (16, 51, 58, 96).

Besides retaining mechanisms for invading new hosts, entering cells, and countering host immune responses, successful facultative symbionts also must affect host phenotypes to enhance the spread and persistence of infected host lines. The nature of these effects is the basis for dividing facultative symbionts into two nonexclusive categories.

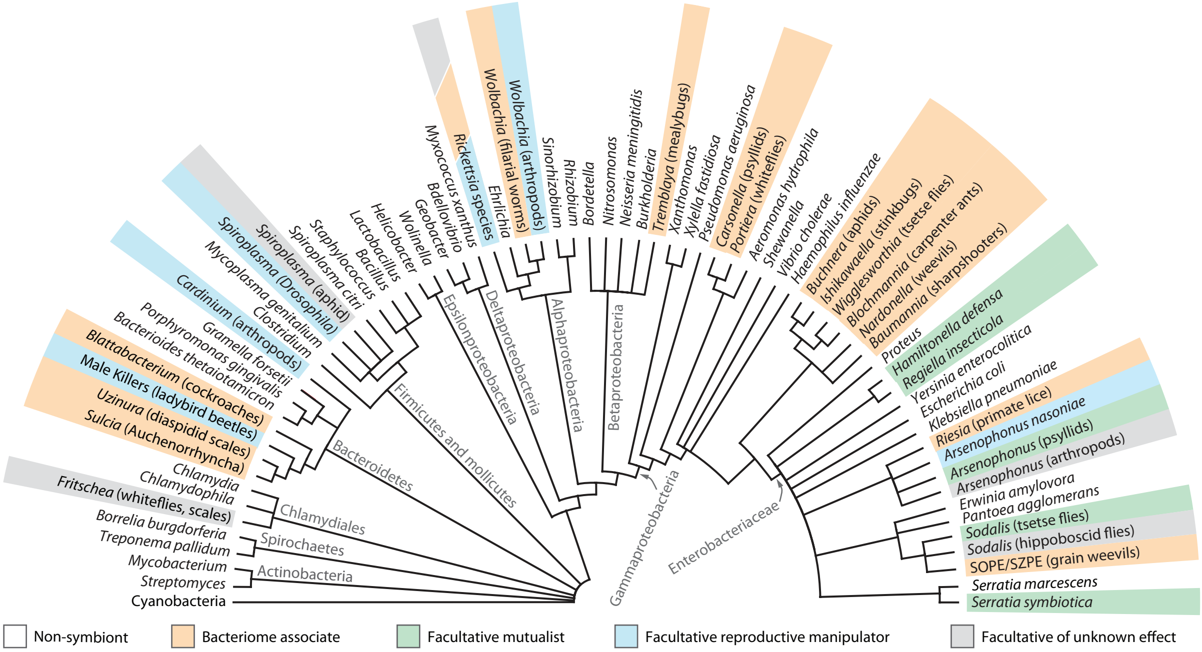
Facultative mutualists confer fitness benefits upon hosts, allowing their carriers to live longer and reproduce more, thereby increasing frequencies of infected hosts. These benefits include protection against natural enemies, heat, or other mortality factors (see below). Bacteria possess a myriad of metabolic and biosynthetic capabilities lacking in insects (and animals generally), so a wide variety of benefits to hosts are possible.

The final category, reproductive manipulators, are parasites that spread by increasing host reproduction through daughters at the expense of reproduction through sons. Their strategies, which reflect the fact that heritable symbionts are usually transmitted maternally, have been reviewed extensively (e.g., 137). One of the most common is reproductive incompatibility between infected and uninfected strains, in which infected males sterilize uninfected females, thereby increasing population frequency of infected matrilines. Other modes of reproductive manipulation are son-killing (which potentially increases investment in daughters), feminization of genetic males, and parthenogenesis. The best-studied reproductive manipulator is *Wolbachia pipientis*, which is widely distributed in arthropods and some other invertebrates and which shows all of these phenotypes (137). Reproductive manipulation has evolved repeatedly in phylogenetically diverse insect heritable symbionts, including *Cardinium hertigii* and other Bacteroidetes (68, 111, 150, 164, 165), *Arsenophonus nasoniae* (Enterobacteriaceae), *Spiroplasma* (74), and *Rickettsia* species (110) (**Figure 1**).

SYMBIONT NOMENCLATURE

Because most symbiotic bacteria are not amenable to laboratory cultivation, they cannot be characterized as required for conferring formal genus and species names under the current rules governing bacterial nomenclature. As a result, most insect symbionts have been named under the *Candidatus* provision for informal naming of genera and species. Exceptions are the few symbionts that have been cultured and a few others named under previous nomenclatural rules (e.g., 8, 19, 25, 55). Another complication affecting symbiont naming concerns the naming of related symbionts isolated due to residence in different host species. In some cases, these have been given a single species designation; thus, *Buchnera aphidicola* is the name for the symbiont in all aphids, although lineages are substantially diverged. In other cases, lineages in different host species are given separate species designations (e.g., 128). Some symbiont lineages have no informal or formal names (e.g., 17, 85). In this review, we use the informal or formal genus name when available; full names can be found in citations.

Heritable pathogens that lower female fecundity, at least in lab assays, do occur (18, 125) but are exceptions, as expected since selection will remove such infected matrilines from populations. In several studies, transferring



"Obligate" symbionts	"Facultative" symbionts	
Bacteriome-associates *Sulcia muelleri* – sharpshooters and other Auchenorrhyncha (87) *Uzinura diaspidicola* – diaspidid scales (60) *Blattabacterium* species – cockroaches (6) *Rickettsia* species – booklice (112) *Tremblaya princeps* – mealy bugs (8) *Carsonella ruddii* – psyllids (100) *Portiera aleyrodidarum* – whiteflies (141) *Buchnera aphidicola* – aphids (8) *Ishikawaella capsulata* – stinkbugs (64) *Wigglesworthia* species – tsetse flies (2) *Blochmannia* species – carpenter ants (56) *Nardonella* species – weevils (82) *Baumannia cicadellinicola* – sharpshooters (161) *Riesia* species – primate lice (3) "SOPE", "SZPE" – grain weevils (17)	**Mutualists** *Hamiltonella defensa* – aphids, whiteflies (96) *Regiella insecticola* – aphids (96) *Arsenophonus* species – psyllids (61) *Sodalis glossinidius* – tsetse flies (143) *Serratia symbiotica* – aphids (96)	**Reproductive manipulators** Male killers – ladybird beetles (85) *Cardinium hertigii* – many arthropods (165) *Spiroplasma* species – butterflies, *Drosophila* (74, 159) *Rickettsia* species – ladybird beetles, wasps, booklice (110) *Wolbachia* species – many arthropods (137) *Arsenophonus nasoniae* – *Nasonia* wasps (55)
	Unknown effect *Fritschea* species – whiteflies, scale insects (46) *Spiroplasma* species – numerous arthropods including aphids, beetles, *Drosophila* (86) *Rickettsia* species – numerous arthropods including aphids, whiteflies, beetles, ticks (110) *Arsenophonus* species – numerous arthropods including aphids (142) *Sodalis* species – hippoboscid flies (102)	

Major properties	Major properties	
Housed within special host organ (bacteriome)	Invades various cells and tissues of hosts	
Long evolutionary history of diversification with host lineages	Short evolutionary history in current host lineage	
No horizontal transfer	Horizontal transfer within and between host species	
Supplies nutrients to hosts	Provides protection against stress or natural enemies	Manipulates reproduction to favor its own host matriline
Extreme genome reduction, <<1 MB	Moderate genome reduction and gene inactivation, >1MB	
Lack of gene uptake, phage, mobile elements or genome rearrangements	Dynamic genomes with bacteriophage, mobile elements, rearrangements	

Figure 1

Evolutionary relationships of heritable endosymbionts of insects and list of major examples and characteristics for each category. The phylogeny is based on widely supported findings from studies listed in the citations. Note that different categories of symbionts, designated by color, cluster within phylogenetic groups.

facultative mutualists or reproductive manipulators to novel hosts results in detrimental effects not observed in the original host (18, 77, 123), indicating that effects on host survivorship and fecundity limit symbiont distributions. Certain benefits of heritable symbionts may be environment dependent; for example, defense against natural enemies will not be observed in lab assays in which those enemies are absent (107). Similar considerations may apply to some other symbionts observed to have detrimental effects in controlled laboratory environments (e.g., 125).

Though most studied symbionts can be placed in one of the three categories of **Figure 1**, frequent apparent shifts and intermediate cases, discussed below, point to commonalities across symbiont lifestyles.

ORIGINS, TRANSMISSION, AND DIVERSIFICATION

Symbiont Origins and Relationships to Free-Living Bacteria

Figure 1 summarizes the phylogenetic distributions of insect heritable symbionts within Bacteria, from combined results of molecular phylogenetic studies that are based on ribosomal RNA sequences (e.g., 8, 60, 64, 82, 96, 97, 108), protein sequences of multiple genes from genome sequencing projects (e.g., 56, 87, 147, 161, 162), or gene content of completely sequenced bacterial genomes (23). Well-supported general results regarding origins of insect symbionts include (*a*) many independent origins from different bacterial phyla and from several lineages within particular phyla; (*b*) concentration of these origins in certain groups, including the Gammaproteobacteria, especially the Enterobacteriaceae, and in the Bacteroidetes; (*c*) evolutionarily derived position of symbionts relative to free-living bacteria.

Although congruent in broad outline, certain phylogenetic results are uncertain and have varied among studies depending on the inclusion of different taxa, methods, and genes. Symbionts show distinctive patterns of gene and genome evolution that complicate the problem of phylogenetic reconstruction, enhancing issues of "long branch attraction" arising from rapid sequence evolution and of convergence, due to shifts in nucleotide base composition. Resolution is not always sufficient to enumerate independent origins of symbiotic lines. The most conspicuous illustration of this issue involves the set of bacteriome-associates of an eclectic group of insect hosts, including *Buchnera* of aphids as well as symbionts of ants, weevils, leafhoppers, and tsetse flies. These sometimes form a clade within the Gammaproteobacteria depending on the phylogenetic analysis (e.g., 23, 82, 161), as depicted in **Figure 1**, raising the question of whether these lineages derive from an ancestor that was already an arthropod symbiont. Weighing against this interpretation are (*a*) possible phylogenetic artifacts resulting from rapid evolution and biased composition of symbiont DNA sequences; (*b*) the disparate composition of the host set, which would imply early horizontal transfers of symbionts, in stark contrast to patterns within particular host groups (see below); and (*c*) the lack of distinctive similarities in genome-level features such as gene set or gene order in comparisons of sequenced genomes. Another cluster of bacteriome-associates, paralleling that mentioned for the *Buchnera*-cluster above, occurs in the Bacteroidetes, including *Blattabacterium* (in cockroaches), *Uzinura diaspidicola* (in scale insects), and *Sulcia* [in Auchenorrhyncha (a sap-feeding insect group including planthoppers, cicadas, spittlebugs, leafhoppers) (60, 87, 138)].

Obligate Mutualist-Host Codiversification and Age of Associations

Codiversification of obligate mutualists and their hosts has now been demonstrated repeatedly for a large variety of insect groups. These results demonstrate that symbiont acquisition is ancient in each case, and followed by vertical transmission that tracks host speciation. The minimum age of infection can be inferred to

Codiversification: strict vertical transmission of symbionts over the course of successive host speciation events, resulting in matching phylogenies be that of the shared ancestor of the infected host group. For many insect lineages, fossils provide indicators of divergence times. Indeed, evidence for symbiont-insect codiversification has provided some of the best cases for estimating dates of bacterial ancestors (e.g., 8, 83, 97).

Buchnera and aphids, the first case for which codiversification was shown (8, 94), provide a typical case history for obligate bacteriome symbiosis. The pattern of codiversification applies at levels from aphids as a whole to matrilines within species (52). The aphid fossil record implies an approximate minimal date of 100–200 million years for the original infection (94). Similar results, supporting ancient infection and codiversification, have since been reported for many other bacteriome-associated symbionts, including those of cockroaches (6, 83), whiteflies, psyllids and mealybugs [reviewed in (8)], diaspidid scale insects (60), the Auchenorrhyncha (97), primate lice (3), tsetse flies (19), weevils (82), and carpenter ants (35, 128).

In each of these cases, symbionts are transmitted maternally, but details of the mechanisms vary as the structures and location of bacteriomes vary (16). The stinkbug example shows that codiversification can be longstanding even for gut symbionts that are not intracellular and that are transmitted externally through maternal deposition on eggs and ingestion by progeny (64).

Evidence for codiversification combined with fossil dating show that bacteriome-associated symbioses are generally extremely old, dating to the origins of numerous major lineages of insect (**Table 1**). The oldest record is 260 million years for the case of *Sulcia*, which infected a shared ancestor of leafhoppers, treehoppers, spittlebugs, cicadas, and planthoppers before the mid-Permian (97). Though fossils are sometimes not sufficient to provide firm dates for ancestral hosts, the overall picture provided by molecular phylogenetic studies of bacteriome-associated symbionts is clear. Symbiosis extends deep into the evolutionary past of a large number of insect groups, and these obligate mutualists can be considered to be stable components of the symbiotic genome in the broad sense.

Loss of Ancestral Symbionts by Host Lineages

Bacteriome-associates are typically required for successful host development and reproduction, raising the question of whether dependence on symbioses represents an evolutionary dead end for host lineages. Several instances of symbiont loss from a host lineage are apparent from

Table 1 Bacteriome-associated symbionts shown to undergo long-term codiversification with hosts and estimated ages of associations

Symbiont group	Hosts	Approximate minimum age[a]	Reference
Buchnera	Aphids	180 My	(94)
Portiera	Whiteflies	180 My	(8)
Carsonella	Psyllids	120 My	(8)
Wigglesworthia	Tsetseflies	>40 My	(19)
Blochmannia	Carpenter ants	50 My	(128)
Baumannia	Sharpshooters	100 My	(138)
Ishikawaella	Stinkbugs	**	(64)
Nardonella	Weevils	**	(82)
Tremblaya	Mealybugs	40 My	(8)
Blattabacterium	Cockroaches	150 My	(83)
Uzinura	Armored scales	100 My	(60)
Sulcia	Auchenorrhyncha	>270 My	(97)

[a]My = Millions of years before present. ** = No fossil-based dates estimated in literature.

molecular phylogenetic studies. Examples include loss of *Buchnera* from a few aphid lineages (49), loss of *Sulcia* from several lineages of sap-feeding insects (16, 97), and loss of the ancestral symbiont *Nardonella* from a family of monocot-feeding weevils (82). In each case, symbiont loss is associated with an apparent replacement by another heritable microorganism. One possible scenario for these replacements is that the ancestral symbiont (obligate mutualist) coexists initially with a more recently acquired organism (facultative mutualist), and that the latter ultimately replaces the more ancient association. A potential example of this has been hypothesized for *Cinara* aphids in which *Buchnera* coexists with more recently acquired symbionts related to *Serratia* that may have replaced some *Buchnera* functions (109). In other cases, coexistence of multiple symbionts is stable over millions of years of host evolution (138).

Replication of bacteriome-associated symbionts is intimately integrated with the early developmental stages of hosts (13, 158, 160). This integration can result in developmental dependence; for example, antibiotic treatment of female aphids generally prevents successful development of embryos (e.g., 16, 157). In the case of *Buchnera*, host dependence may be overcome in certain cases. Presence of *Serratia symbiotica* sometimes enables pea aphids (*Acyrthosiphon pisum*) to reproduce following experimental elimination of *Buchnera* although this replacement does not occur in all pea aphid genotypes in the lab (77) and has not been observed outside the lab. Also, in some aphid species, males and specialized sterile soldiers do not contain *Buchnera*, whereas reproductive females do, demonstrating that developmental dependence on symbionts is not always insurmountable even in a long established obligate symbiosis (16, 49).

Distribution and Origins of Facultative Symbionts

Facultative symbionts often occur at low titers in hosts and show irregular distributions among host tissues and species. As a result, assessing their prevalence depends on molecular methods, and their abundance has been even less appreciated than that of obligate symbionts. In fact, they are almost certainly more common. Diagnostic PCR screens reveal *Wolbachia* infection in at least 20% of insect species (73, 155) and *Cardinium* in about 6% (165). Though these estimates are rough (as they depend on sampling and the particular PCR primers used), these two symbionts are clearly widespread in arthropods.

Facultative symbionts, especially those from Enterobacteriaceae, appear to be particularly common in hosts that possess bacteriome-associated obligate symbionts, including aphids, whiteflies, tsetse, and mealybugs. Whether this reflects more intensive study or greater frequency of occurrence is not clear. Immune responses are major obstacles to colonizing and persisting in hosts [reviewed in (146)], and bacteriocytes may provide a haven from immune responses (58). Differences in immune function among insect taxa may explain why some are more often colonized by certain symbiont groups. For example, a screen of 35 *Drosophila* species for heritable symbionts, based on universal PCR for bacterial rRNA genes using DNA from dissected ovaries of female flies, revealed only *Wolbachia* and *Spiroplasma* (86), in contrast to the much higher frequency of heritable symbionts from Enterobacteriaceae found in sap-feeding insect groups.

As for bacteriome-associated symbionts, facultative symbionts originate from a variety of ancestral bacterial groups, including some of the same groups producing bacteriome-associates (**Figure 1**). Rickettsiales, Mollicutes, and Chlamydiae, all host-restricted groups originally known as vertebrate pathogens, are being found to contain many symbionts of arthropods and other invertebrate hosts (e.g., 24, 45, 46, 58, 110, 151), raising the possibility that most basal diversity in these groups consists of symbiotic organisms, with pathogens being atypical and derived. The Enterobacteriaceae has generated numerous facultative symbionts, based on studies in various insects (8, 20, 29, 55, 96, 102, 122, 126, 152).

Horizontal Transfer of Facultative Symbionts Within and Between Host Species

A variety of observations show that facultative symbionts undergo horizontal transfer among matrilines within and between species (e.g., 108, 126). While the bacteriome-associates of a particular host group consistently form a strongly supported clade (such as *Buchnera* in aphids), facultative symbionts typically vary in presence among members of the same or related host species. Many studies show erratic distribution of *Wolbachia* and *Cardinium* (108, 137, 155, 165). Numerous insect groups contain a single bacteriome-associate descending from an ancient infection, but several different secondary symbionts with erratic distributions suggest multiple transfers and losses [for examples, see 8)]. Furthermore, facultative symbionts are often shared between divergent host groups; for example, *Hamiltonella* and *Arsenophonus* species are found in scattered species of aphids, psyllids, whiteflies, planthoppers, and other groups (8, 25, 55, 122, 126, 131). In tsetse, another member of the Enterobacteriaceae, *Sodalis*, is universally present, as is the obligate mutualist, *Wigglesworthia*, but *Sodalis* undergoes transfer within and between host species (152).

Although molecular phylogenetic studies imply past horizontal transfer among matrilines, this transfer is not often observed in lab experiments. As a result, the routes by which new matrilines are colonized are largely elusive. An exception is the case of the aphid facultative mutualists, *Hamiltonella* and the related *Regiella insecticola*, in which intraspecific transfer between matrilines can occur during mating, via paternal transfer to females and to their progeny, resulting in stable infections that are subsequently transferred maternally (92). Paternal transfer has not been found for other heritable symbionts and appears to be absent in reproductive manipulators (as expected for symbionts with phenotypes favoring female transmission), based on both lab and population studies.

Transmission via natural enemies or other ecological interactions has been proposed but has only rarely been observed in experiments. Two exceptions are the finding that parasitic mites can move *Spiroplasma* between *Drosophila* species (71) and that *Wolbachia* can be transferred between species of parasitic wasps developing in the same host individual (67). For aphids, the most readily plausible routes of interspecific transfer, through food plants or parasitoid wasps, are either absent or rare enough that they are not observed in lab experiments (32; K.M. Oliver, unpublished data). Nonetheless, intra- and interspecific transmission via an assortment of ecological contacts seems widely possible since experimental infections are readily achieved in the lab, through either microinjection (18, 105, 116, 124) or ingestion of diets containing symbiont cells (32, 116). The likelihood of successful transfer is enhanced if infection capability persists in symbionts outside their hosts, as observed for *Wolbachia*, which can infect even after a week of isolation from host cells (117).

Although horizontal transfer within and between species appears to be frequent for these symbionts, their distributions are far from random. Many instances are known in which a particular insect group shows a high frequency of a particular facultative symbiont, due to multiple acquisitions and transfers. For example, *Arsenophonus* has repeatedly invaded whiteflies (142) but is rare in aphids (122), which frequently harbor other facultative symbionts from Enterobacteriaceae (96, 126). *Wolbachia* appears rare in aphids (122, cf. 57) but is common in many other groups (20, 72, 73, 84, 155).

Horizontal transfer among hosts can produce coinfections within hosts and opportunity for recombination and gene transfer. Thus, even if infrequent, it has potentially massive consequences for symbiont population structure and genome dynamics, as discussed further below.

Evolutionary Transitions: Deleterious to Beneficial and Facultative to Obligate

Several bacterial clades consist of a mix of obligate and facultative symbionts (**Figure 1**), indicating that transitions between facultative and obligate symbionts have occurred. This pattern suggests that obligate bacteriome associates evolve from heritable facultative symbionts that lose horizontal transmission capabilities. For example, *Arsenophonus* comprises facultative symbionts that are sometimes reproductive manipulators and harmful to hosts (55) and sometimes likely beneficial but facultative in hosts (8, 61, 122); in contrast, *Riesia*, the sister clade to *Arsenophonus*, contains the obligate, bacteriome-associated symbionts of sucking lice (3). Another closely related cluster includes *Sodalis*, facultative symbionts of tsetse and other bloodsucking flies (102), plus the bacteriome-associated symbionts of both grain weevils and of chewing lice, two unrelated insect groups (50, 63). Similarly, the Rickettsiales, the host-restricted clade containing *Wolbachia*, *Rickettsia*, and *Ehrlichia*, shows a spectrum of interactions with hosts, ranging from pathogenic to obligatory for host survival and development (31, 110, 112). Although it is usually a reproductive manipulator or facultative symbiont of unknown effect in arthropod hosts, *Wolbachia* can be beneficial to insect hosts (34) and is an obligate symbiont of filarial nematodes (140). *Rickettsia* itself includes obligate symbionts associated with bacteriomes of booklice (112) as well as widespread facultative symbionts of which some are reproductive manipulators (110). *Rickettsia*, *Wolbachia*, or both, are required for normal oogenesis in some bark beetles (163).

Shifts from reproductive manipulator to beneficial symbiont can be rapid. For example, in populations of *Drosophila simulans* in California, an invading *Wolbachia* was transformed in less than 25 years from a reproductive manipulator that reduced fecundity of female carriers to a mutualist that enhanced fecundity (149). Such transitions reflect the strong selection on heritable symbionts for traits that increase productivity of the host matriline.

GENOME EVOLUTION IN SYMBIOTIC BACTERIA

Genomic Features of Obligate Mutualists

Striking shared features are evident in the numerous complete genome sequences now available for bacteriome-associated symbionts of insects (**Table 2**). The most prominent is extreme genome reduction, reflected in small chromosomes with few genes. Other characteristics in common are biased nucleotide base composition, favoring adenine and thymine (A+T), and rapid sequence evolution, resulting in accelerated rates of amino acid substitution in all genes. In these features, bacteriome-associates of insects are representative of a more widespread syndrome found in host-restricted symbionts and pathogens such as the Mollicutes, the Rickettsiales, the Chlamydiales, and others. The rapid evolution, A+T bias, and effects on protein evolution were detectable before full genomes were available (90). Genome reduction became evident with the first symbiont genome sequenced, for *Buchnera* of pea aphids (134), and was subsequently found to be the norm for bacteriome-associates (153). Known genomes of obligate insect bacteriome-associates range from 160–800 kb and 16.5%–33% G+C (**Table 2**). In contrast, related free-living bacteria often have genomes more than five times larger (usually 2–8 Mb) and with base compositions nearer to 50% G+C.

Reflecting their rapid sequence evolution, obligate symbionts essentially always appear on long branches of phylogenetic trees (e.g., 21), and statistical (relative rates) tests repeatedly indicate acceleration of symbiont sequence evolution both for the commonly sequenced 16S rRNA gene and for protein-coding genes throughout the genome (60, 82, 90, 136, 139, 147). Substitution rates are particularly elevated at sites affecting amino acid replacements within coding genes, as reflected in increased

Table 2 Features of sequenced genomes of heritable insect symbionts

	Buchnera				***Blochmannia***	
	APS	**Sg**	**Bp**	**Cc**	***floridanus***	***pennsylvanicus***
Genome size (kb)	641	641	616	416	706	792
GC content (%)	26	25	25	20	27	29
Coding region (%)	86	83	81	85	83	76
rRNA gene set	1	1	1	1	1	1
Protein-coding genes	564	546	504	357	583	610
Replication, recombination, and repair	42	37	37	30	31	31
Transcription	19	18	17	17	21	22
Translation	120	117	117	114	116	114
Cell wall/membrane biogenesis	30	25	26	5	53	58
Amino acid transport and metabolism	61	60	57	42	74	75
Coenzyme transport and metabolism	36	34	24	7	45	51
Reference	(134)	(139)	(147)	(109)	(56)	(36)
	Wigglesworthia	***Baumannia***	***Carsonella***	***Sulcia***	***Sodalis***	***Wolbachia wMel***
Genome size (kb)	698	686	160	246	4171	1268
GC content (%)	22	33	17	22	54	35
Coding region (%)	86	85	97	96	50	80
rRNA gene set	2	2	1	1	7	1
Protein coding genes	611	595	182	227	2432	1195
Replication, recombination, and repair	30	41	5	8	150	110
Transcription	22	24	5	6	185	34
Translation	109	119	56	74	156	127
Cell wall/membrane biogenesis	61	34	1	6	155	46
Amino acid transport and metabolism	35	55	33	47	199	45
Coenzyme transport and metabolism	65	64	3	10	98	42
Reference	(2)	(161)	(100)	(87)	(143)	(162)

rates of changes affecting amino acid relative to changes at silent codon positions (90, 154). The combination of biased amino acid composition and rapid evolution results in proteins with lower predicted thermal stability (147).

The gene sets of bacteriome-associated symbionts are largely subsets of the gene repertoires of related free-living bacteria. Though foreign gene uptake has been rampant in bacterial evolution generally, it appears to have been rare in the evolution of obligate bacterial symbionts (36, 87, 109, 139, 161). In particular, the acquisition of special "symbiosis" genes is not evident. Instead, the establishment of symbiosis appears to occur through modification of ancestral bacterial genes and through coadaptation of host genes. A caveat here is that most sequenced symbiont genomes are highly derived and reduced to near-minimal levels (**Table 2**). They may have eliminated genes initially required in the evolution of symbiosis but deleted after the evolution of host-based mechanisms to maintain symbionts.

The reduced genomes lack genes in almost all functional categories, with relatively high retention of those involved in essential processes such as translation, replication, and transcription. DNA repair genes are depleted in all of these genomes, but the particular repair genes retained vary (**Table 2**, **Figure 2**). In most cases, many genes required for production of cell envelope components are missing

(**Table 2**, **Figure 2**) (109, 134, 139); these are especially depleted in *Carsonella* and *Sulcia* (87, 100). Symbionts enclosed in a host-derived membrane within bacteriocytes (*Buchnera*, *Sulcia*, *Carsonella*) lose a greater proportion of genes involved in production of the cellular envelope than do symbionts that are free in the cytosol (*Wigglesworthia*, *Blochmannia*), suggesting that host functions can replace those of the original bacterial cell envelope. Genome reduction suggests the occurrence of intimate exchange between symbiont and host (99). Unusual close associations are also supported by some other observations, for example, bacterial symbionts living within host mitochondria or host nuclei (4, 127), or bacterial symbionts dwelling within another type of bacterial symbiont in the host cytoplasm (148).

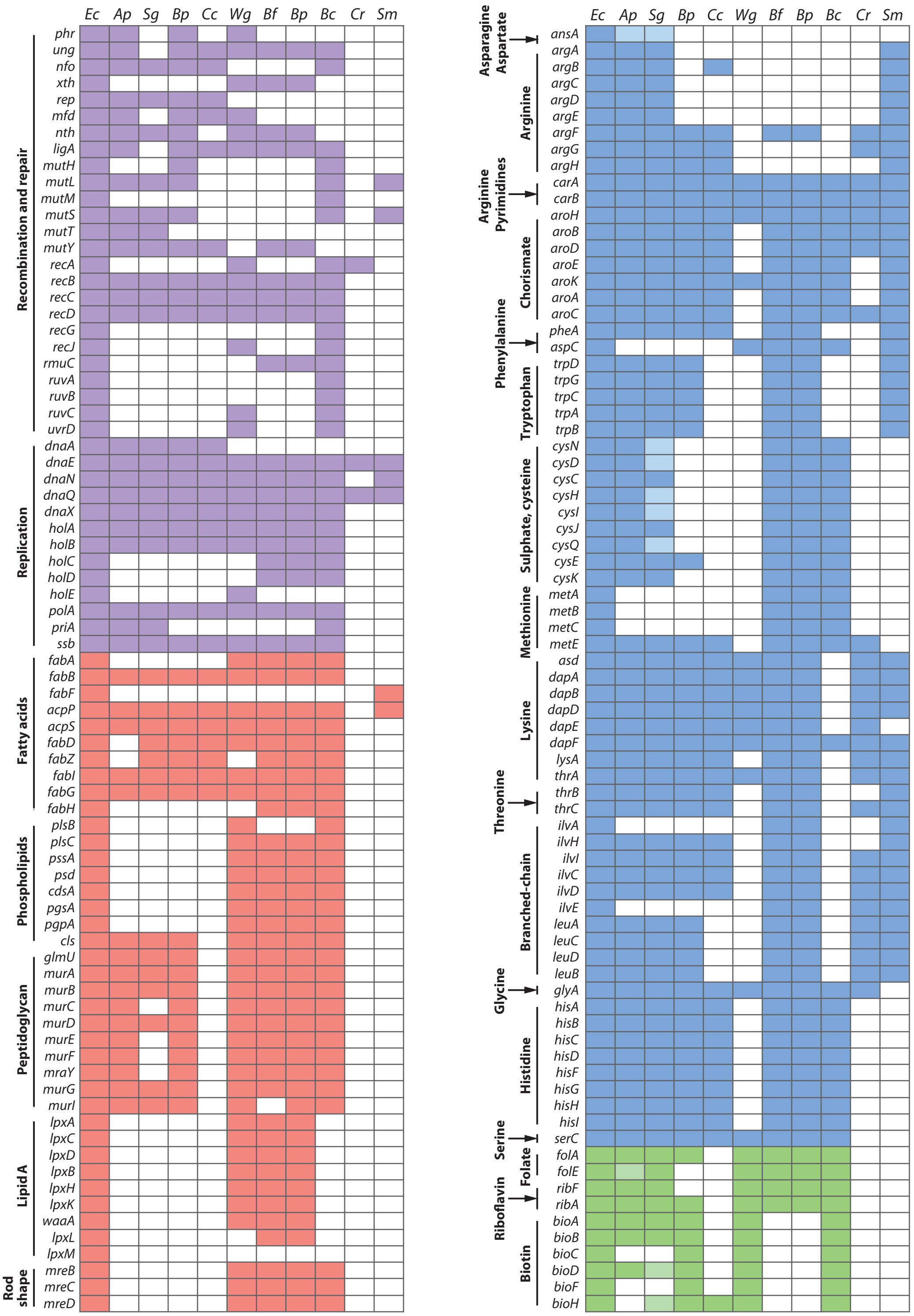
Genomes of bacteriome-associates show a consistent shift toward elevated A+T content, resulting in the most extreme biases in base composition known (**Table 2**). This bias, also found in obligate pathogenic bacteria such as Rickettsiales and Chlamydiales, reflects mutational bias favoring A+T, and is strongest at sites that are neutral or near neutral with respect to selection, such as silent positions in codons or in intergenic spacers. In *Buchnera*, for example, the silent sites and spacer base compositions are less than 10% G+C, whereas overall genome composition is between 25% and 30% G+C for *Buchnera* genomes. Nonetheless, the bias also has a major effect on amino acid composition of proteins (36, 90, 100, 147). Mutational bias is likely to reflect the loss of DNA repair pathways. Supporting this possibility, *Baumannia* shows the least bias (33% G+C) and retains more repair genes, whereas *Carsonella* and *Sulcia* show the most bias (16.5% and 22%) and retain almost no repair genes.

Another prominent feature of bacteriome-associates, likely linked to the lowered thermal stability of proteins, is the constitutively elevated expression of heat shock proteins. High expression was noted for chaperonin (GroEL protein) in *Buchnera* and other obligate symbionts (48). Once genome sequences were available, microarray and quantitative RT-PCR studies showed that other heat shock proteins also show unusually high expression in the absence of stress (93, 156). This overexpression is likely a compensatory adaptation that moderates effects of lower protein stability resulting from mutations genome-wide (47, 90, 147).

Extreme Genomes

Two obligate insect symbionts represent the extremes among known cases of genome reduction. The first is the obligate symbiont of psyllids, *Carsonella* (Gammaproteobacteria), which has a genome that is both the smallest and the most biased in base composition reported to date for cellular organisms (**Table 2**) (100). The other is the obligate symbiont of leafhoppers and relatives, *Sulcia* (Bacteroidetes) with the second smallest known genome (**Table 2**) (87). Both have lost many genes considered to be essential (**Figure 2**), raising the as-yet unresolved question of how these cells continue to replicate and function (87, 100).

Facultative symbionts: dynamic genomes. Based on partial or full genome sequences for *Sodalis glossinidius* from tsetse flies (143), *Wolbachia w*Mel from *Drosophila melanogaster* (162), and *Hamiltonella* from aphids (91), facultative symbionts exhibit radically different genome features from those of long-term bacteriome associates. Whereas the latter lack mobile elements, bacteriophage, and most repetitive DNA, and generally have few pseudogenes, genomes of facultative symbionts are larger, have many repetitive regions and unusually high numbers of mobile elements, and contain phage and phage-derived genes. For example, the 4.2-Mb genome of *Sodalis* contains many insertion sequences and bacteriophage-derived sequences (22, 33) as well as a remarkable number of ancestral genes undergoing degradation as pseudogenes, yielding a coding density of only 50%, among the lowest known for bacterial genomes (143). *Wolbachia* also possesses phage-derived sequences and repetitive elements, though it has a more compact genome (1.3 Mb) with few pseudogenes (54, 162).



		Ec	Ap	Sg	Bp	Cc	Wg	Bf	Bp	Bc	Cr	Sm
Recombination and repair	phr	■	■	□	■	□	■	□	□	□	□	□
	ung	■	■	□	■	■	■	■	■	■	□	□
	nfo	■	■	■	■	■	□	□	□	■	□	□
	xth	■	□	□	□	□	■	■	■	□	□	□
	rep	■	■	■	■	■	□	□	□	□	□	□
	mfd	■	■	□	■	■	■	□	□	□	□	□
	nth	■	■	■	■	□	■	■	■	□	□	□
	ligA	■	■	□	■	■	■	■	■	■	□	□
	mutH	■	□	□	■	□	□	□	□	■	□	□
	mutL	■	■	■	■	□	□	□	□	■	□	■
	mutM	■	□	□	□	□	□	□	□	■	□	□
	mutS	■	■	■	■	□	□	□	□	■	□	■
	mutT	■	■	■	□	□	□	□	□	□	□	□
	mutY	■	■	■	■	■	□	■	■	□	□	□
	recA	■	□	□	□	□	■	□	□	■	■	□
	recB	■	■	■	■	■	■	■	■	■	□	□
	recC	■	■	■	■	■	■	■	■	■	□	□
	recD	■	■	■	■	■	■	■	■	■	□	□
	recG	■	□	□	□	□	□	□	□	■	□	□
	recJ	■	□	□	□	□	■	□	□	■	□	□
	rmuC	■	□	□	□	□	□	■	■	■	□	□
	ruvA	■	□	□	□	□	□	□	□	■	□	□
	ruvB	■	□	□	□	□	□	□	□	■	□	□
	ruvC	■	□	□	□	□	■	□	□	■	□	□
	uvrD	■	□	□	□	□	■	□	□	■	□	□
Replication	dnaA	■	■	■	■	■	□	□	□	□	□	□
	dnaE	■	■	■	■	■	■	■	■	■	■	■
	dnaN	■	■	■	■	■	■	■	■	■	□	■
	dnaQ	■	■	■	■	■	■	■	■	■	■	■
	dnaX	■	■	■	■	■	■	■	■	■	□	□
	holA	■	■	■	■	■	■	■	■	■	□	□
	holB	■	■	■	■	■	■	■	■	■	□	□
	holC	■	□	□	□	□	□	■	■	■	□	□
	holD	■	□	□	□	□	□	■	■	■	□	□
	holE	■	□	□	□	□	■	□	□	□	□	□
	polA	■	■	■	■	■	■	■	■	■	□	□
	priA	■	■	■	□	□	□	□	□	■	□	□
	ssb	■	■	■	■	■	■	■	■	■	□	□
Fatty acids	fabA	■	□	□	□	□	■	■	■	■	□	□
	fabB	■	■	■	■	■	■	■	■	■	□	□
	fabF	■	□	□	□	□	□	□	□	□	□	■
	acpP	■	■	■	■	■	■	■	■	■	□	■
	acpS	■	■	■	■	■	■	■	■	■	□	□
	fabD	■	□	■	■	■	■	■	■	■	□	□
	fabZ	■	□	■	■	■	□	■	■	■	□	□
	fabI	■	■	■	■	■	■	■	■	■	□	□
	fabG	■	■	■	■	■	■	■	■	■	□	□
	fabH	■	□	□	□	□	□	■	■	■	□	□
Phospholipids	plsB	■	□	□	□	□	■	□	□	■	□	□
	plsC	■	□	□	□	□	■	■	■	■	□	□
	pssA	■	□	□	□	□	■	■	■	■	□	□
	psd	■	□	□	□	□	■	■	■	■	□	□
	cdsA	■	□	□	□	□	■	■	■	■	□	□
	pgsA	■	□	□	□	□	■	■	■	■	□	□
	pgpA	■	□	□	□	□	■	■	■	■	□	□
	cls	■	■	■	■	□	■	■	■	■	□	□
Peptidoglycan	glmU	■	■	■	■	□	■	■	■	■	□	□
	murA	■	■	■	■	□	■	■	■	■	□	□
	murB	■	■	■	■	□	■	■	■	■	□	□
	murC	■	■	□	■	□	■	■	■	■	□	□
	murD	■	■	■	■	□	■	■	■	■	□	□
	murE	■	■	□	■	□	■	■	■	■	□	□
	murF	■	■	□	■	□	■	■	■	■	□	□
	mraY	■	■	□	■	□	■	■	■	■	□	□
	murG	■	■	■	■	□	■	■	■	■	□	□
	murI	■	■	■	■	□	■	□	■	■	□	□
Lipid A	lpxA	■	□	□	□	□	■	■	■	□	□	□
	lpxC	■	□	□	□	□	■	■	■	□	□	□
	lpxD	■	□	□	□	□	■	■	■	□	□	□
	lpxB	■	□	□	□	□	■	■	■	□	□	□
	lpxH	■	□	□	□	□	■	■	■	□	□	□
	lpxK	■	□	□	□	□	■	■	■	□	□	□
	waaA	■	□	□	□	□	■	■	■	□	□	□
	lpxL	■	□	□	□	□	□	■	■	□	□	□
	lpxM	■	□	□	□	□	□	□	□	□	□	□
Rod shape	mreB	■	□	□	□	□	■	■	■	■	□	□
	mreC	■	□	□	□	□	■	■	■	■	□	□
	mreD	■	□	□	□	□	■	■	■	■	□	□

		Ec	Ap	Sg	Bp	Cc	Wg	Bf	Bp	Bc	Cr	Sm
Asparagine Aspartate →	ansA	■	▨	▨	□	□	□	□	□	□	□	□
Arginine	argA	■	■	■	□	□	□	□	□	□	□	■
	argB	■	■	■	□	■	□	□	□	□	□	■
	argC	■	■	■	□	□	□	□	□	□	□	■
	argD	■	■	■	□	□	□	□	□	□	□	■
	argE	■	■	■	□	□	□	□	□	□	□	■
	argF	■	■	■	■	■	□	■	■	□	■	■
	argG	■	■	■	■	■	□	□	□	□	■	■
	argH	■	■	■	■	■	□	□	□	□	□	■
Arginine Pyrimidines →	carA	■	■	■	■	■	■	■	■	■	■	■
	carB	■	■	■	■	■	■	■	■	■	■	■
	aroH	■	■	■	■	■	■	■	■	■	■	■
Chorismate	aroB	■	■	■	■	■	□	■	■	■	■	■
	aroD	■	■	■	■	■	□	■	■	■	■	■
	aroE	■	■	■	■	■	□	■	■	■	□	■
	aroK	■	■	■	■	■	■	■	■	■	□	■
	aroA	■	■	■	■	■	□	■	■	■	□	■
	aroC	■	■	■	■	■	□	■	■	■	■	■
Phenylalanine →	pheA	■	■	■	■	■	□	■	■	□	□	■
	aspC	■	□	□	□	□	■	■	■	■	□	■
Tryptophan	trpD	■	■	■	■	□	□	■	■	□	□	■
	trpG	■	■	■	■	□	□	■	■	□	□	■
	trpC	■	■	■	■	□	□	■	■	□	□	■
	trpA	■	■	■	■	□	□	■	■	□	□	■
	trpB	■	■	■	■	□	□	■	■	□	□	■
Sulphate, cysteine	cysN	■	■	▨	□	□	□	■	■	■	□	□
	cysD	■	■	▨	□	□	□	■	■	■	□	□
	cysC	■	■	■	□	□	□	■	■	■	□	□
	cysH	■	■	▨	□	□	□	■	■	■	□	□
	cysI	■	■	▨	□	□	□	■	■	■	□	□
	cysJ	■	■	■	□	□	□	■	■	■	□	□
	cysQ	■	■	▨	□	□	□	■	■	■	□	□
	cysE	■	■	■	■	□	□	■	■	■	□	□
	cysK	■	■	■	□	□	□	■	■	■	□	□
Methionine	metA	■	□	□	□	□	□	■	■	■	□	□
	metB	■	□	□	□	□	□	■	■	■	□	□
	metC	■	□	□	□	□	□	■	■	■	□	□
	metE	■	■	■	■	■	□	■	■	■	■	□
Lysine	asd	■	■	■	■	■	■	■	■	□	■	■
	dapA	■	■	■	■	■	■	■	■	□	■	■
	dapB	■	■	■	■	■	■	■	■	□	■	■
	dapD	■	■	■	■	■	■	■	■	□	■	■
	dapE	■	■	■	■	■	■	■	■	□	■	□
	dapF	■	■	■	■	■	■	■	■	■	■	■
	lysA	■	■	■	■	■	□	■	■	□	■	■
	thrA	■	■	■	■	■	■	■	■	□	■	■
Threonine →	thrB	■	■	■	■	■	□	■	■	□	□	■
	thrC	■	■	■	■	■	□	■	■	□	■	■
Branched-chain	ilvA	■	□	□	□	□	□	■	■	□	□	■
	ilvH	■	■	■	■	■	□	■	■	□	□	■
	ilvI	■	■	■	■	■	□	■	■	□	■	■
	ilvC	■	■	■	■	■	□	■	■	□	■	■
	ilvD	■	■	■	■	■	□	■	■	□	■	■
	ilvE	■	□	□	□	□	□	■	■	□	■	■
	leuA	■	■	■	■	□	□	■	■	□	■	■
	leuC	■	■	■	■	□	□	■	■	□	■	■
	leuD	■	■	■	■	□	□	■	■	□	■	■
	leuB	■	■	■	■	□	□	■	■	□	■	■
Glycine →	glyA	■	■	■	■	■	■	■	■	■	■	□
Histidine	hisA	■	■	■	■	■	□	■	■	■	□	□
	hisB	■	■	■	■	■	□	■	■	■	□	□
	hisC	■	■	■	■	■	□	■	■	■	□	□
	hisD	■	■	■	■	■	□	■	■	■	□	□
	hisF	■	■	■	■	■	□	■	■	■	□	□
	hisG	■	■	■	■	■	□	■	■	■	□	□
	hisH	■	■	■	■	■	□	■	■	■	□	□
	hisI	■	■	■	■	■	□	■	■	■	□	□
Serine →	serC	■	■	■	■	■	■	■	■	■	□	□
Folate	folA	■	■	■	■	□	■	■	■	■	□	□
	folE	■	▨	■	□	□	■	■	■	■	□	□
Riboflavin →	ribF	■	■	■	□	□	■	■	■	■	□	□
	ribA	■	■	■	■	□	■	■	■	■	□	□
Biotin	bioA	■	■	■	■	□	■	□	□	■	□	□
	bioB	■	■	■	■	□	■	□	□	■	□	□
	bioC	■	□	□	■	□	■	□	□	■	□	□
	bioD	■	■	▨	■	□	■	□	□	■	□	□
	bioF	■	□	□	■	□	■	□	□	■	□	□
	bioH	■	□	▨	■	■	■	□	□	■	□	□

Massive proliferation of mobile elements is found in the bacteriocyte associates of grain weevils, which are closely related to *Sodalis* (115) and which appear to be relatively recently derived as bacteriome-associates (82). Based on the few multilocus studies to date, facultative symbionts show varying extents of interstrain recombination and gene transfer (5, 9, 37). Some genome features typical of obligate symbionts are observed at less extreme levels in facultative symbionts, including elevated sequence evolution, gene loss, and shift toward higher A+T content (91, 96, 162).

Causes of distinctive features in symbiont genomes. Why do heritable symbionts, as well as other obligately host-restricted bacteria, consistently show reduced genomes, with rapidly evolving sequences, A+T-bias, unstable proteins, and elevated expression of heat shock genes? The striking similarities in independently evolved obligate symbiont genomes indicate that common forces are at work. Symbionts encounter elevated genetic drift resulting from relaxed selection on many genes and from radical change in population structure that results in lowered efficacy of selection even on required genes (47, 90, 139, 147, 153). Whereas free-living bacteria can have very large genetic population sizes, enabling selection to act on very slight differences in fitness, obligate restriction to hosts and bottlenecks at the stage of inoculation of new hosts (e.g., 88, 158) result in a greater role of genetic drift. The consequence is a greater rate of fixation of mildly deleterious mutations, including amino acid changes that lower stability of proteins, and inactivation and loss of nonessential genes, and mutation to less-preferred codons. Additional observations supporting the role of genetic drift in these genomes is the limited evidence for adaptive codon bias (154) and low levels of polymorphism within species (1, 53).

Among nonessential genes that are eliminated are DNA repair genes (**Figure 2**), and these losses will result in elevation of the effective mutation rate itself. Such elevation is suggested by the strong mutational bias favoring A+T, discussed above. Estimates of per-site mutation rates calibrated indirectly also support elevated mutation in obligate symbionts (103).

Genomes of facultative symbionts and recently derived symbionts such as those in grain weevils also exhibit features that are consistent with elevated levels of genetic drift, due to reduced genetic population size. This can cause dispensable genes to be degraded and insertion sequences to proliferate unchecked by selection (115). Ultimately, mobile elements will be eliminated in clonally evolving symbiont lineages (95), as will pseudogenes that gradually shrink through deletions (89).

Theoretical studies on mutation fixation in heritable mutualistic symbionts have demonstrated that such genomes can experience elevated genetic drift and increased rates of fixation of mildly deleterious mutations (104, 113, 121, 135). Fixation of mutations deleterious to fitness is increased when horizontal transmission among hosts is decreased or eliminated (104). Depending on whether selection on a particular locus acts within hosts or between hosts, both the size of the symbiont inoculum during infection and the size of the host population can be important determinants of the rate of mutation fixation. Some models

Genetic drift: the effect of chance on allele frequencies (most important for small populations or for genes under weak selection)

Figure 2

Gene content of reduced genomes from obligate bacterial symbionts. Boxes representing genes in replication, recombination, and repair are colored violet; cell envelope synthesis and cell shape are orange; amino acid synthesis are blue; and cofactor synthesis are green. Pseudogenes are indicated by a lighter shade. Pathways for biosynthetic processes are shown in their linear order as best as possible. The representations of cofactor biosynthesis pathways are severely limited due to space. GenBank accession numbers for the genomes are NC_000913 (*Escherichia coli*, abbreviated *Ec* in the figure), NC_002528 (*Buchnera*-Ap, Ap), NC_004061 (*Buchnera*-Sg, Sg), NC_004545 (*Buchnera*-Bp, Bp), NC_008513 (*Buchnera*-Cc, Cc), NC_004344 (*Wigglesworthia glossinidia*, *Wg*), NC_005061 (*Blochmannia floridanus*, *Bf*), NC_007292 (*Blochmannia pennsylvanicus*, *Bp*), NC_007984 (*Baumannia cicadellinicola*, *Bc*), NC_008512 (*Carsonella ruddii*, *Cr*), and NC_010118 (*Sulcia muelleri*, *Sm*).

show that mutational decay need not result in complete dysfunction of symbionts, as host-level selection potentially imposes a limit beyond which further deleterious mutations are effectively eliminated (113). This result provides a solution to one of the most frequent questions about long-term mutualistic symbiosis: Do symbiont genomes undergo relentless degradation resulting in mutual extinction or the requirement that the symbiont be replaced? The antiquity of many symbioses and the current ecological success of many animal hosts with very old symbioses would suggest that extinction is not inevitable. In part, this is likely due to the evolution of compensatory mechanisms, on the part of the host or on the part of the symbiont.

Aposymbiotic: descriptive of individuals of the host species that have been deprived of their symbionts, through experimental treatment or natural forces

Adaptation in Symbiont Genomes

Although insect nutritional symbionts rely primarily upon ancestral gene repertoires for making products needed by hosts and although much evidence indicates that mutation and drift have greater evolutionary impact in symbionts than in free-living relatives, this does not imply that adaptation is absent. Instances of apparent adaptation to improve provisioning of host nutrients are the plasmid-associated amplification of genes for biosynthesis of tryptophan (80) and leucine (12) in *Buchnera*. Additionally, evolutionary sequence analyses aimed at detecting the action of natural selection on particular genes have provided evidence for adaptive evolution in surface molecules, transporters and other genes in *Wolbachia* (15, 75) and of the highly expressed GroEL protein in bacteriome-associates (48).

INFERRING FUNCTIONS OF SYMBIONTS FROM MOLECULAR STUDIES

Some of the major insights from genome sequencing of symbionts have involved the functional capabilities of symbionts and of their contributions to host biology. These insights have been especially fruitful for nutrient-provisioning obligate mutualists.

Nutritional Roles of Obligate Bacteriome-Associated Symbionts

Buchner (16) proposed that the function of bacteriome-associated symbionts was the provision of nutrients. This role has been impressively confirmed and elaborated by genomic studies. Prior to molecular studies, *Buchnera* was thought to provide hosts with essential amino acids rare or absent from the host diet, phloem sap, based on experimental evidence from studies using defined diets and aposymbiotic aphids (40). Starting with cloning and sequencing of genes underlying numerous amino acid biosynthetic pathways (7) and continuing with the full genome sequencing of several primary symbionts including *Buchnera* from four aphid species (**Figure 1**), DNA sequence data have repeatedly revealed intact or near-intact pathways for nutrients likely to be needed by the hosts (**Figure 2**), despite extreme reductions in gene repertoires in these small genomes. Some insects, including carpenter ants, have more variable diets in nature, with the result that the role of the symbionts is less readily identified. But in insects with narrowly defined diets, such as strict sap-feeders and blood-feeders, symbiont genomes encode pathways for amino acids and vitamins that fit closely with expected nutritional needs [reviewed in (166)].

One advantage of full genome sequences over partial sequence information is the identification of enzymes and pathways that are lacking in an organism; this can potentially disprove conjectures about possible roles of symbionts. For example, the proposal that aphid primary symbionts provision sterols (8) can be rejected (40): *Buchnera* resembles other Gammaproteobacteria in lacking genes for sterol production.

For cases in which two or more genomes are available, including *Buchnera*, *Blochmannia*, and the symbionts of *Calyptogena* clams, comparative analyses show that gene loss continues even in extremely reduced genomes (36, 79, 139). Gene categories that continue to be eroded fall within several functional categories, including transcriptional regulators, DNA repair genes, genes for synthesis of cell surface components, and biosynthetic pathways linked to host nutrition. Thus, among the four sequenced *Buchnera* genomes, the smaller two have lost genes underlying several amino acid biosynthetic pathways, and in *Buchnera* of *Schizaphis graminum*, genes in the pathway for fixation of inorganic sulfur are present but inactivated as evidenced by several nonsense codons and frameshift mutations, indicating an early evolutionary stage following the loss of this capability (139) (**Figure 2**). Likewise, the two sequenced *Blochmannia* species differ in biosynthetic capabilities relevant to host nutrition, with only one retaining the pathway for synthesis of coenzyme A (36). In the extreme case of the sequenced strain of *Carsonella*, numerous amino acid biosynthetic pathways are missing enzymes for particular steps (100), in contrast to the largely intact pathways found in *Sulcia* and other small genome symbionts (87) (**Figure 2**). Since the sequenced *Carsonella* strain appears to be the sole symbiont in its host, one explanation is that this insect can enhance the supply of nutrients provisioned by plants, as known for some other sap-feeders (e.g., 81).

Multiple Symbionts Within a Host

Dependence on multiple obligate heritable symbionts is found in numerous insects, reflecting successive acquisition during evolution of host lineages. Most cases are little studied. The most complete picture of a dual symbiosis is that for sharpshooters, in which genomes of both symbionts have been sequenced. One symbiont, *Sulcia*, represents the more ancient association and is present in an extended set of related plant-sucking insects (97). The other, *Baumannia*, is restricted to sharpshooters. Both symbionts have codiversified with their hosts over a period representing millions of years (97, 138). Based on genome sequencing for the two symbionts of the glassy-winged sharpshooter (*Homalodisca vitripennis*), these organisms show striking complementarity in abilities to provision particular nutrients to hosts, indicating that both are obligate mutualists (87, 161) (**Figure 2**). As noted above, *Sulcia* is heavily devoted to amino acid production and encodes enzymes for synthesis of all amino acids required as animal nutrients with the exception of the eight steps of the histidine pathway and the final four steps of methionine production. *Baumannia* lacks amino acids biosynthetic pathways with the striking exceptions of all eight steps of histidine biosynthesis and the four genes for the conversion of homoserine to methionine. In contrast to *Sulcia*, the *Baumannia* genome encodes pathways for production of numerous cofactors (87). Sharpshooters feed on the particularly nutrient-deficient diet of xylem sap, and acquisition of *Baumannia* may have been a critical step in the origin of xylem-feeding in this insect lineage (87, 97).

A possible early stage in the establishment of a dual symbiosis is found in the aphid *Cinara cedri*, in which *Buchnera* possesses an especially reduced genome (109) (**Table 2**). Among genes present in other *Buchnera* but lost in *Buchnera* of *C. cedri* are those encoding the pathway for the synthesis of tryptophan, a required nutrient. *C. cedri* possesses another heritable symbiont, related to *Serratia* species, which encodes the pathway for tryptophan biosynthesis. Thus, dependence on this *Serratia* may have evolved to furnish required nutrients no longer provisioned by the more ancient symbiont (109). More recently acquired symbionts might sometimes entirely replace ancestral symbionts (82). However, this possibility would be prevented if each symbiont becomes indispensable through complementary retention of needed pathways, as in the case of *Baumannia* and *Sulcia* in sharpshooters.

Insights into Function in Facultative Symbionts

What functions are essential in facultative symbionts of insects? Whereas bacteriocyte-associates can be considered passive domesticates of their hosts, which have evolved to maintain and transmit them (16, 99), facultative symbionts must rely on their own devices for

Type Three Secretion Systems (TTSSs): molecular syringes used by many pathogenic bacteria for secreting proteins that target host cells and enable entry

Transfection: the transfer and establishment of symbionts from an original host to a new host of the same or different species

invading hosts and overcoming host immune responses. Once a facultative symbiont has infected a host, it must attain a stable titer sufficient to persist and infect progeny but not so high that hosts die or fail to reproduce. And it must exert some effect on the host phenotype that causes the symbiont to spread rather than to decline in frequency in the host population.

Molecular studies combined with experimental work have begun to reveal possible mechanisms through which facultative symbionts achieve some of these ends [for overview, see (27)]. Although facultative symbionts are more difficult to harvest in large numbers directly from hosts, some of them offer the advantage that they can be grown in pure culture or within insect cell lines (25, 30, 39, 55), and most can be transferred to novel host lines, opening a range of experiments not possible with bacteriocyte-associates (116). Nonetheless, the obstacles to linking sequence to function are substantial (69, 116).

Host cell invasion presents the same obstacles for an intracellular bacterium whether it is pathogen or symbiont, and homologous mechanisms are used (27). As the most striking example, Type Three Secretion Systems (TTSSs) are required for establishment of symbiosis by the facultative symbiont of tsetse, *Sodalis*, based on experimental work with mutant strains (26, 28). Similar situations seem to occur in other symbionts within the Enterobacteriaceae, including the facultative symbiont *Hamiltonella* (91) and the relatively recently evolved bacteriome-associates of grain weevils (“SOPE” and “SZPE”) (28). TTSSs appear to be involved in establishing symbiosis in all known facultative symbionts in Enterobacteriaceae (27).

As noted above, particular combinations of host species and facultative symbionts appear to be incompatible, based on transfection experiments (e.g., 123). Such incompatibilities contribute to the nonrandom patterns of associations, in which certain insect groups repeatedly acquire symbiont types that are rare in other groups. So far, the molecular basis for this host specificity is not clear.

Facultative Symbionts: Strategies

Once established in a host and able to infect progeny, the next challenge for a heritable symbiont is to increase the frequency of infected matrilines within the host population. To this end, facultative symbionts show a remarkable array of strategies, though so far these are only tentatively linked to particular genes and pathways (27, 69). In both facultative mutualists and reproductive manipulators, it has been proposed that an ability to influence host phenotype is linked to phage or mobile elements associated with host genomes (10, 11, 37, 91).

Facultative mutualists enhance their own hosts’ reproduction, thus infecting more progeny. Based on experimental work, a particularly frequent benefit appears to be the provision of protection against natural enemies of the host species (105, 106, 107, 114, 129). In some cases, the basis for protection is unknown, but sequenced-based studies have revealed that it is sometimes likely based in the production of a toxin deterrent that is associated with bacteriophage (37, 114). Other effects include protection against heat stress (18, 124) and possibly nutritional benefits (109).

Reproductive manipulators adopt strategies resulting in reproductive abnormalities that increase the frequency of infected matrilines. The most intensive study has focused on *Wolbachia* (69). Densities of the symbiont, which affect expression of the cytoplasmic-incompatibility phenotype in hosts, have been proposed to be under the control of the *Wolbachia* bacteriophage, WO (10). *Wolbachia*-specified features direct host cell machinery to achieve particular patterns of localization in oocytes, an essential step in achieving efficient vertical transmission (132).

HERITABLE SYMBIONTS AS DYNAMIC ELEMENTS IN HOST ECOLOGY

All categories of heritable symbionts—obligate bacteriome-associates, facultative mutualists, and reproductive manipulators—can vary within species and populations in ways that impact host development and reproduction and that affect host evolution, even on short time scales (118, 119). Although bacteriome-associates are generally obligate and universally present in a host species, their genomes are subject to mutations with repercussions for host fitness. A recent example of this was documented for *Buchnera* in pea aphids; a single base deletion in the promoter of the small heat shock gene (*ibpA*) resulted in temperature-dependent effects on fitness (41). Aphid lines with symbionts bearing the mutation had higher fitness at moderate temperatures but severely compromised fitness when subjected to heat stress, relative to lines with symbionts lacking the mutation. Furthermore, the mutation arises repeatedly and can reach high frequencies in *Buchnera* within aphid populations. *Buchnera* genomes from different aphid hosts vary in presence of particular amino acid pathways (**Figure 2**), and such variation may underlie differences in nutritional requirements that are known within aphid species.

For facultative mutualists, host fitness can be heavily dependent on infection by a particular strain or by presence/absence of a symbiont. For example, pea aphids show variation in ability to resist attack by parasitoids, and this variation can depend on infection by particular strains of *Hamiltonella* with no influence of aphid genotype (106). In competition trials, *Hamiltonella*-infected lines outcompete uninfected lines when parasitoids are present but are eliminated when parasitoids are absent (107). Symbiont-based fitness differentials are also known for *Serratia symbiotica* and *Regiella*, which affect the ability to withstand heat (124), to use particular host plants (144), or to resist pathogenic fungi (129). The dependence of these symbiont-conferred benefits on environmental conditions provides an explanation for variable symbiont frequencies in host populations. Maternal inheritance often approaches 100% (92, 126); thus, if symbionts were uniformly beneficial, one would expect them to be fixed in populations and species.

Reproductive manipulators, such as *Wolbachia*, potentially enhance rates of speciation of hosts, by creating barriers to gene flow, a potential noted in the initial reports of microbe-mediated reproductive incompatibility (e.g., 14). Several studies on closely related insect species or populations verify that reproductive manipulators can promote reproductive isolation and thus potentially contribute to the high species diversity of insects (11, 70). These processes can impact host population structure and evolution, resulting in the sweep of a single matriline through an entire species or in the long-term maintenance of multiple matrilines through frequency-dependent processes acting on symbionts (e.g., 42, 44, 76). Reproductive manipulators can be highly dynamic over short time scales, resulting in rapid increases in frequency or transformations from reproductive manipulator to mutualist, as exemplified by *Wolbachia* in *Drosophila* populations and species (118, 149).

Because facultative symbionts can have profound short-term impacts on host ecology and can affect host interactions with other organisms in the environment (65), they potentially can be exploited in efforts to manipulate pest populations (120).

Perhaps the most radical impact of insect symbionts on the ongoing evolution of their hosts is their potential role as portals of gene acquisition. Recent work shows that facultative symbionts can be acquired as stable elements, conferring new capabilities and that, once acquired, facultative symbionts can take up novel genes borne by bacteriophage (10, 37). Facultative symbionts can therefore extend the effective genetic repertoire of the host. Furthermore, numerous studies on *Wolbachia* and insect genomes have demonstrated that fragments of symbiont genomes can be incorporated into the host insect genome (66, 78, 101), although so far none has demonstrated that these transferred genes are being expressed or display a phenotype. Thus, symbiotic associations enable genes specialized in the context of one host and environment to be introduced into novel host species.

CONCLUSIONS

Insect symbionts are critical players in all aspects of host ecology, reproduction and evolution. Genome-level studies on these diverse bacteria have revealed some of the extremes of genome evolution, and have illustrated the rapidity with which changes in lifestyle can evolve in bacteria.

Despite the great progress in understanding the roles and dynamics of symbionts in insect populations, new research has produced at least as many questions as answers. Answering these questions will require drawing on diverse fields, from genomics, cell biology, biochemistry to evolutionary biology and ecology.

SUMMARY POINTS

1. Maternally transmitted insect symbionts are widespread, and include obligate nutritional mutualists, facultative mutualists that protect against natural enemies or stress, and reproductive manipulators that enhance productivity of infected female lines.
2. Insect symbionts originated independently from a number of major bacterial groups, especially Gammaproteobacteria and Bacteroidetes.
3. Obligate bacteriome-associated symbionts are typically ancient and have coevolved with major insect lineages.
4. Phylogenetic clusters include symbionts with different effects on hosts, and transitions between reproductive manipulators and mutualists appear to be common.
5. Genomes of heritable symbionts display features expected under long-term evolution with increased levels of genetic drift due to small population size.
6. Genomes of some obligate insect mutualists are extremely tiny, with gene sets smaller than what was previously considered possible.
7. Obligate mutualists have static genomes, but facultative symbiont genomes are dynamic and contain mobile elements, bacteriophage, and evidence for recombination.
8. Both obligate and facultative symbionts have ongoing impact on host ecology and evolution, possibly promoting speciation, affecting ecological tolerances, and enabling the uptake of foreign genes that impact ecological processes.

FUTURE ISSUES

1. Insect symbionts provide the most extreme examples of genome reduction, and their ability to function with so few genes is still unexplained.
2. Facultative symbionts are now recognized as ubiquitous, but the biological effects of most are unknown.
3. Findings that facultative symbionts can be mutualistic as defensive agents imply that their roles will be understandable only in the context of complex natural environments.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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