Invertebrate Symbioses

Remaining Flexible in Old Alliances: Functional Plasticity in Constrained Mutualisms

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Central to any beneficial interaction is the capacity of partners to detect and respond to significant changes in the other. Recent studies of microbial mutualists show their close integration with host development, immune responses, and acclimation to a dynamic external environment. While the significance of microbial players is broadly appreciated, we are just beginning to understand the genetic, ecological, and physiological mechanisms that generate variation in symbiont functions, broadly termed "symbiont plasticity" here. Some possible mechanisms include shifts in symbiont community composition, genetic changes via DNA acquisition, gene expression fluctuations, and variation in symbiont densities. In this review, we examine mechanisms for plasticity in the exceptionally stable mutualisms between insects and bacterial endosymbionts. Despite the severe ecological and genomic constraints imposed by their specialized lifestyle, these bacteria retain the capacity to modulate functions depending on the particular requirements of the host. Focusing on the mutualism between *Blochmannia* and ants, we discuss the roles of gene expression fluctuations and shifts in bacterial densities in generating symbiont plasticity. This symbiont variation is best understood by considering ant colony as the host superorganism. In this eusocial host, the bacteria meet the needs of the colony and not necessarily the individual ants that house them.

Introduction

SYMBIOTIC INTERACTIONS PROFOUNDLY affect the life history, physiology, and adaptation of all organisms. Among the most pervasive symbioses are those between a bacterial partner and a eukaryotic host. While the best-studied microbial associates are still disease-causing parasites, recent work illustrates the profound importance of commensal and mutualistic interactions, ranging from astonishingly diverse microbiota of gastrointestinal tracts, to simpler interactions with one or few symbiotic partners. By combining the biochemical functions of two domains of life, the establishment of bacterial-eukaryotic mutualisms often represent key evolutionary innovations (Margulis and Fester, 1991), allowing partners to thrive in niches and utilize resources that otherwise would be inaccessible.

In addition to their significance in evolutionary transitions, beneficial microbes are closely tied to host responses on physiological and ecological timescales. The function of symbiotic populations or communities can vary considerably, depending on host developmental stage (Chun *et al.*,

2008), temperature (Baird *et al.*, 2009), immune responses (Wen *et al.*, 2008), resource availability (Zahran, 1999), host diet (Ley *et al.*, 2008), or drug treatments (Dethlefsen *et al.*, 2008). It is clear that microbes play important roles in mediating physiological changes across host life stages and environmental contexts. To understand how organisms develop, grow, maintain health, and respond appropriately to an ever-changing environment, we must consider the symbiotic players involved.

The role of symbiosis in the host's dynamic life history is a key but complex area of study. By what mechanisms do symbiont functions vary within hosts, including shifts in community composition, gene expression patterns, or symbiont densities? Understanding these mechanisms sets the stage to address the more difficult question of whether observed changes are meaningful to the host. That is, do changes in microbial symbionts reflect coordinated responses that benefit the host, selfish phenotypes in a stressed interaction, or neutral consequences of an environmental perturbation? The answers to these questions will surely depend on the age and integration of the association, the environmental

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variation encountered, and the genetic and ecological processes at work. By exploring symbiont plasticity in a variety of systems, we can begin to understand the range of possible responses of microbes to hosts and the general principles guiding those responses.

In any set of comparisons, the extremes can be especially informative. Among beneficial microbes, the long-term mutualists of insects engage in exceptionally stable and constrained interactions. As rare examples of intracellular mutualists, these bacteria are closely integrated into host development and nutritional physiology, reflecting long-term coevolution (Buchner, 1965). Like any organism, insects housing endosymbionts do not live in a static environment; hosts grow, age, and cope with environmental variation. In social insects, such as ants, the host colony develops and matures through the activities of different castes. What roles do insect symbionts play across these types of variation? Given their long evolutionary history in a constrained environment, these mutualists might lack certain mechanisms for genetic and functional variation found in more labile symbiotic interactions. On the other hand, functional plasticity may be all the more important in otherwise stable associations.

In this review, we explore the impact of symbiotic lifestyle on genetic variation and the ability of microbes to respond to distinct host life stages or environmental contexts. We cite examples from a range of insect endosymbionts, and focus on a long-term ant mutualist whose host might be considered the colony itself. Despite the severe evolutionary and genomic constraints imposed upon insect mutualists, recent work and our own data illustrate that the bacteria retain the capacity to modulate functions in response to particular requirements of the host.

Lifestyle Constraints in Insect Mutualists

Among multicellular organisms, insects as a group form exceptionally diverse associations with microbes, including viruses, protists, bacterial pathogens, and facultative bacterial endosymbionts that form labile associations. In addition, many insects possess bacterial mutualists that live exclusively within specialized host cells (bacteriocytes) and female reproductive organs. In contrast to intracellular pathogens and facultative secondary symbionts, these long-term primary endosymbionts are considered essential for host survival and reproduction (Buchner, 1965; Ishikawa, 2003). Primary mutualists often occur in insects that feed on nutritionally unbalanced diets, such as plant sap (e.g., aphids and psyllids), vertebrate blood (tsetse flies and lice), or grains (weevils). By supplying key nutrients missing in these diets, the bacteria allow insects to exploit food sources that would otherwise be inadequate (Baumann et al., 2000; Akman-Gündüz and Douglas, 2009). Unlike environmentally acquired symbionts, primary mutualists undergo maternal transmission to offspring. The congruence of host and bacterial phylogenies points to cospeciation for tens or hundreds of millions of years (e.g., Munson et al., 1991; Thao et al., 2000; Baumann and Baumann, 2005).

Interestingly, primary mutualists also occur in insects that feed on complex diets, such as ants in the tribe Camponotini and cockroaches (Buchner, 1965). These bacteria are thought to play more general nutritional roles involving nitrogen recycling and other functions. As a model to explore symbiont plasticity in a long-term mutualism, our labs focus on the ant mutualist called Candidatus Blochmannia (Blochman, 1882; Sauer et al., 2000). The bacteria are located in ant ovaries, consistent with maternal transmission to host offspring, and within bacteriocytes that are intercalated among enterocytes of the ant midgut (Buchner, 1965; Dasch, 1975; Sauer et al., 2002). Blochmannia has been studied most extensively in Camponotus, the second largest ant genus with ~1000 species worldwide. Sometimes termed carpenter ants because many species excavate nests in wood, its nesting habits also include live trees and shrubs, within soil beneath rocks, and the rainforest canopy where Camponotus and other ant genera can comprise up to 94% of arthropods and 86% of the biomass (Davidson et al., 2003). Phylogenetic analyses indicate a single infection of Blochmannia at least before the divergence of three genera in the Camponitini >30 MYA and cospeciation since that time (Sameshima et al., 1999; Sauer et al., 2000; Degnan et al., 2004).

Stability of Symbiont Lineages

In interactions involving multiple symbionts, such as the microbiota of GI tracts, shifts in symbiont community profiles provide a potential mechanism for functional plasticity. By contrast, such shifts are not a major source of variation for most insect mutualists, due to their stability and exceptionally low genetic diversity. With some notable exceptions (see below), primary mutualists often consist of one bacterial lineage in a given host group. Moreover, the bacteria within a host individual are genetically homogeneous or very similar, due to continual diversity-purging population bottlenecks upon each inoculation of developing host eggs or embryos (Buchner, 1965; Mira and Moran, 2002).

Stable mutualisms involving multiple bacterial lineages occur in insects, but few are well characterized. The best studied is a dual, or coprimary, nutritional endosymbiosis in sharpshooters (Takiya et al., 2006). In addition, so-called secondary symbionts have infected mealybugs several times independently and coevolve with hosts, although over shorter time periods than the primary symbiont (Thao et al., 2002). Replacement of one mutualist lineage with a distinct microbial species has occurred in weevils (Conord et al., 2008) and aphids (Fukatsu and Ishikawa, 1996) and might play an important role over evolutionary timescales. Thus, changes in the profiles of mutualists are possible in a very limited sense, either due to shifts in the relative abundance of two mutualists, or lineage replacements over evolutionary time.

If we define the symbiotic community of insects more broadly, to also include diverse facultative associates, then shifts in the composition of a "super symbiotic system" (Koga et al., 2003) may be quite significant as the host responds to environmental conditions. These secondary symbionts are common in some insect groups, such as aphids, psyllids, and tsetse flies. The bacteria transfer frequently across species and help hosts cope with abiotic and biotic factors. For instance, aphid secondary symbionts increase tolerance to high temperatures (Montllor et al., 2002; Russell and Moran, 2006), reduce rates of fungal infection (Scarborough et al., 2005), confer resistance to parasitoids (Oliver et al., 2003; Moran et al., 2005b; Hansen et al., 2007; Degnan

and Moran, 2008), and play a role in host plant preferences (Tsuchida *et al.*, 2004). These bacteria might also complement the nutritional functions of primary mutualists. For example, secondary symbionts compensate for the loss of primary mutualists in the lab, presumably due to nutritional functions provided (Koga *et al.*, 2003). In addition, tryptophan biosynthesis genes missing from the tiny (422 kb) genome of a *Buchnera* strain (Perez-Brocal *et al.*, 2006) are encoded by the secondary symbiont that is abundant in the same aphid species, suggesting that both symbionts are necessary to provision this essential amino acid (Gosalbes *et al.*, 2008). In this sense, facultative endosymbionts expand the potential for evolutionary innovation and may catalyze the formation of new, primary endosymbioses.

Lifestyle Constraints Reflected in the Genome

Genome degradation in insect mutualists

The constrained lifestyle of stable mutualists not only suppresses their genetic variation within hosts, but also has pervasive effects on patterns of DNA sequence and genome evolution (Moran, 1996; Andersson and Kurland, 1998; Moran *et al.*, 2008). Across phylogenetically independent lineages, transitions to this lifestyle are coupled with apparent genetic degradation, including accelerated rates of molecular evolution, reduced genomic $G + C$ content, as well as severe genome reduction (Fig. 1). This syndrome of genetic degradation may, in part, result from a reduction in effective population sizes (N_e), as these bacteria undergo successive bottlenecks when transmitted to host offspring (Andersson and Kurland, 1998; Funk *et al.*, 2001; Abbot and Moran, 2002). According to the nearly neutral theory of evolution (Ohta, 1973), reduced N_e is expected to increase the rate of fixation of deleterious mutations by random genetic drift. Over time, the accumulation of deleterious mutations could negatively affect the fitness of the symbiont and its host.

Gene loss in insect mutualists is thought to reflect a combination of factors. Due to an underlying deletion bias in bacteria (Mira *et al.*, 2001), genes persist only if selection favors their maintenance. Within a host cellular environment, genes encoding metabolic diversity and other functions may become redundant and lost under relaxed selection. In addition, selection to maintain genes will be less effective in these mutualists, due to reduced N_e. Fast nucleotide muta-

tion rates and strong AT bias also drive gene disruption and loss. A recent population genomic study of the aphid mutualist, *Buchnera*, showed that replication errors within polyA tracts (abundant in AT-rich genomes) introduce frameshifts and trigger the process of gene erosion (Moran *et al.*, 2009). Gene transfer to the host nucleus is also a very real possibility, as suggested by EST data for aphids (Nakabachi *et al.*, 2005) and the absence of numerous critical functions in mutualist genomes, particularly the 160-kb genome of a psyllid symbiont (Nakabachi *et al.*, 2006).

Despite severe gene loss in most functional categories, endosymbionts retain machinery for their own basic cellular processes (e.g., transcription, translation, and replication) and specific functions that benefit the host (e.g., amino acid or vitamin biosynthetic genes, as well as urease genes in *Blochmannia* of ants). Conspicuous losses in these mutualists include the absence of mobile DNA, the deletion of *recA* from *Buchnera* and *Blochmannia*, and the absence of the replication gene *dnaA* from *Wigglesworthia* of tsetse flies (Akman *et al.*, 2002) and from *Blochmannia* of ants (Gil *et al.*, 2003; Degnan *et al.*, 2005). Relating to functional plasticity, mutualists have lost gene regulation functions to varying degrees (see Appendix). For a comparison of gene contents, we point the reader to recent reviews on this topic (Zientz *et al.*, 2004; Moran *et al.*, 2008; Feldhaar and Gross, 2009).

Genome stability in insect mutualists

Typically, bacterial evolution involves extensive genome fluidity, including lateral gene transfer, inversions, and translocations. Among parasitic microbes, horizontal transfer of pathogenicity islands and antibiotic resistance genes has indisputable significance. Likewise, genome fluidity generates diversity in many beneficial microbes. For example, the transfer of symbiotic loci on plasmids or genomic islands influences nitrogen-fixing rhizobia (Mergaert *et al.*, 1997), and phage-mediated transfer of toxin genes shapes the defensive phenotype of aphid secondary symbionts (Degnan and Moran, 2008).

These major sources of genetic innovation for most bacteria—the acquisition of foreign DNA and shuffling of existing genes—is unavailable in at least some long-term mutualists. Instead, gene deletions are irreversible and may constrain the evolutionary potential of host and symbiont alike. Primary mutualists show no signs of horizontal gene transfer, with the notable exception of plasmid movement in some *Buchnera* lineages (Van Ham *et al.*, 2000; Gil *et al.*, 2006). For genera that have had more than one genome sequenced, the genome dynamics within the context of a long-term association can be evaluated. Such studies have found striking genome stability in *Buchnera* (Tamas *et al.*, 2002) and *Blochmannia* (Degnan *et al.*, 2005), implying no inversions or translocations during the tens of millions of year of evolution within hosts. Genome differences within a mutualist group are dominated by differential gene loss. This overall lack of gene acquisition or shuffling may reflect the loss of recombination genes and mobile DNA, combined with limited opportunities to encounter genetically distinct bacteria.

Gene Expression Variation

The increased accessibility of functional genomic approaches has opened new doors to explore gene expression
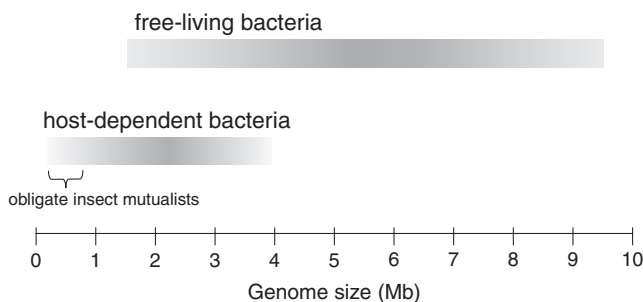
FIG. 1. Genome size ranges for obligately host-associated and free-living bacterial species. Host-dependent bacteria typically undergo severe genome reduction. Primary mutualists of insects include the smallest bacterial genome known, the 160 kb chromosome of *Carsonella* associated with psyllids (Nakabachi *et al.*, 2006).

in various symbiotic systems. Studies using microarrays, subtractive hybridization, EST sequencing, and meta-transcriptome analysis are illuminating the role of transcriptional plasticity in symbiotic interactions. As a starting point, many studies begin with the bacterial partner, for which genomic data are often available first. Ideally, expression analysis will include both partners in the symbiosis, to detect coordinated responses. The development of microarrays with host and symbiont loci is a promising avenue for this work (Wilson *et al.*, 2006).

Among insect endosymbionts, gene expression began with the striking observation that symbionts produce almost exclusively one protein, called symbionin, which we now know is encoded by *groEL* (Ishikawa, 1984; Baumann *et al.*, 1996). Since that time, we have learned that *groEL* is constitutively overexpressed in many other symbioses (Aksoy, 1995; Charles *et al.*, 1997; Fares *et al.*, 2004). This chaperonin may help to stabilize proteins that accumulate deleterious changes in the small populations of host-dependent microbes (Moran, 1996). More recently, functional genomic studies have illustrated reduced levels of gene expression differences in primary mutualists compared to *Escherichia coli* and other free-living bacteria. Studies in *Buchnera* shows high expression levels of certain chaperonins and amino acid biosynthetic genes, but very modest transcriptional responses when the host is exposed to heat shock (Wilcox *et al.*, 2003) or changes in dietary amino acid content (Moran *et al.*, 2005a). The loss of regulatory genes may limit the ability of long-term mutualists to alter transcriptional patterns in response to environmental fluctuations.

In the *Blochmannia*–*Camponotus* association, the ecology and physiology of hosts may be particularly variable and enhance selection for plasticity of endosymbiont functions. The bacterium must not only operate in different developmental stages, but also function in different castes and through the entire life cycle of an ant colony. Hymenopteran castes represent the most extreme morphological, biochemical, and behavioral variation ever documented among genetically equivalent organisms (Evans and Wheeler, 2001). Ants and their colonies tend to be especially long-lived, with workers typically living several months and the queens living several years to decades (Hölldobler and Wilson, 1990). As workers age, they change behaviorally and physiologically as they progress from working within nests to foraging. Two distinct types of workers, minors and majors, are biased to certain tasks and have physiologies to match. Queens are radically different than workers, as they are specialized to begin new colonies and have heavy nutritional demands to support egg production (Wheeler and Buck, 1995). The variety of food exchanges in ant colonies implies that the interaction of *Blochmannia* and an individual host will affect other individuals and the colony as a whole.

A key difference in the *Blochmannia*–ant mutualism in comparison to those in nonsocial insects is the reduced impact of symbiont loss to the individual host. Ant workers survive antibiotic treatment and in many respects appear to be normal. For example, elimination of *Blochmannia* with rifampicin had no effect on worker mortality (Feldhaar *et al.*, 2007), and antibiotic treatment of other stages does not result in death (the authors' unpublished data). These patterns make sense if we consider the ant colony as a superorganism (Hölldobler and Wilson, 2009). The effects of symbionts on

this superorganism involve nutrient levels that will be distributed to the colony members with the greatest nutrient demand. The symbionts meet the needs of the colony, not necessarily the individual ants that house them. Individuals can carry out resource-intensive processes above the level found in solitary insects, because the symbiont products synthesized in many individual ants can flow toward larvae or queens, greatly enhancing their performance. As predicted under this framework, the major difference between workers with versus without *Blochmannia* involved the ability of these workers to rear brood (Feldhaar *et al.*, 2007).

Given this exceptional variation at the host level, there is much interest in mechanisms that underlie functional variation in *Blochmannia* across host life stages and castes. In a recent microarray study, Stoll *et al.* (2009) tracked changes in *Blochmannia* transcription across distinct developmental stages and worker ages for the host species *Camponotus floridanus*. Shifts in *Blochmannia* gene expression were modest and typically fell below a threefold change, although several (~20) genes showed greater than fivefold variation in expression level. This overall reduction in expression fluctuations is similar to earlier findings in *Buchnera*, and might reflect the loss of many regulation genes in both endosymbionts (Wilcox *et al.*, 2003; Moran *et al.*, 2005a).

While insect mutualists are missing many gene regulation functions, they vary in the extent of this loss (Table 1). For example, *Blochmannia* of *Camponotus pennsylvanicus* (i.e., *Blochmannia pennsylvanicus*) diverged from *Blochmannia floridanus* ~20 MYA and retains key regulatory genes that *B. floridanus* has lost. These distinct regulatory functions include *hns*, encoding the histone-like nucleoid-structuring protein (H-NS) that functions as a major bacterial chromatin component and can play an important role in global regulation; *ispH* involved in control of the stringent response that allows bacteria to survive conditions of amino acid starvation; *hfq*, a pleiotropic posttranscriptional regulator; *mntR*, a DNA-binding transcriptional repressor; and *yfgB* involved in posttranscriptional modification 23S rRNA. Having retained these regulatory capabilities, it is possible that *B. pennsylvanicus* shows a greater capacity for gene expression fluctuations, an hypothesis we are currently testing.

Symbiont Density Fluctuations

In symbionts with limited gene regulation capabilities, shifts in bacterial densities may offer an important mechanism for plasticity. Many studies have shown dramatic differences in the densities of primary mutualists within hosts, with numbers depending upon host developmental stage, sex, or age (Wolschin *et al.*, 2004; Kono *et al.*, 2008), host nutrition (Feldhaar *et al.*, 2007; Wilkinson *et al.*, 2007), and stressors such as predation (Mahadav *et al.*, 2008).

It is often difficult to distinguish whether symbiont proliferation and decline represent adaptive responses that benefit the host, or alternatively, a breakdown in the normal regulation of symbiont numbers. In *Camponotus*, *Blochmannia* densities vary widely throughout development and show hints of an adaptive response. Symbionts reach their highest densities in pupae undergoing metamorphosis and in young imagines (Wolschin *et al.*, 2004; Stoll *et al.*, 2009). At these stages, high metabolic requirements include sclerotization and melanization of the cuticle and may require increased

synthesis of amino acids (especially tyrosine), nitrogen recycling, and other symbiont nutritional functions. Further supporting the hypothesis that symbiont proliferation benefits hosts, the same host stages show a modest increase in the relative expression of symbiont nutritional functions (Zientz et al., 2004; Stoll et al., 2009). Interestingly, Blochmannia densities declined in workers fed a diet lacking essential amino acids (Feldhaar et al., 2007). While one might predict that increased symbiont load could help to compensate for dietary deficiencies, bacterial replication probably imposes some cost to the host. Apparently, workers deprived of essential nutrients lack the resources to support high symbiont densities. Notably, the unusual absence of dnaA in Blochmannia might provide the host some control over replication.

Transcriptional Slippage as a Potential Mechanism for Plasticity

Transcription in AT-rich endosymbionts may be influenced by the long, homopolymeric tracts (especially polyA regions) that are common in protein-coding genes. Such tracts are generally considered deleterious because they are prone to slippage errors during replication (which may disrupt the reading frame, as noted above). In addition, RNA polymerase errors along these tracts may introduce frameshifts in transcripts, producing a heterogeneous pool of mRNA transcripts with varied numbers of A's in the homopolymeric region. When this happens, some fraction of transcripts will contain frameshifts and fail to encode full-length proteins. An accurate picture of gene expression profiles in AT-rich symbionts will depend on understanding whether such transcriptional slippage is prevalent.

As a case study, Tamas et al. (2008) tested for transcriptional slippage in several Buchnera and Blochmannia genes that contain long polyA tracts. cDNA libraries showed variable tract lengths, indicating a high prevalence of RNA polymerase slippage. While transcriptional slippage typically disrupts the reading frame of an intact gene, it also restores the reading frame in transcripts of presumed pseudogenes. At genes that contain frameshifts in the genomic DNA, 12–50% of transcripts contained corrected reading frames that could potentially yield full-length proteins. Oddly, a surprising conservation of frameshifts within such genes suggests that they are maintained by selection over evolutionary time (Tamas et al., 2008).

Although speculative and difficult to test, it is possible that endosymbionts with reduced regulatory capabilities might exploit transcriptional slippage to alter the abundances of full-length proteins, achieving an indirect form of transcriptional plasticity. A DNA frameshift within a polyA tract could reduce (but thanks to transcriptional slippage, not eliminate) the production of intact transcripts and full-length proteins. Replication slippage that corrects the reading frame in the genomic DNA would be a very simple back-mutation that restores the higher expression level. In this sense, the persistence of error-prone tracts could offer a fortuitous avenue for plasticity. In any event, when quantifying transcription profiles in symbionts with homopolymeric tracts, it will be important to consider that the effective expression level of loci with polyA tracts depends on the prevalence of transcriptional slippage.

Conclusions

Symbioses vary in their complexity, from the highly diverse microbiota of GI tracts, to relatively simple, binary associations. These distinct lifestyles affect the genetic diversity of microbial symbionts and their capacity to respond to variation at the host level. At an extreme end of the spectrum, the specialized lifestyle of long-term insect mutualists severely constrains the genetic and metabolic diversity of these bacteria. Once established within a host group, these bacteria specialize at the expense of their abilities to acquire new functions or to shuffle existing genes. While one might imagine that gene expression variation could be especially important under such circumstances, these bacteria have lost many regulatory genes in the course of extensive genome reduction. As predicted, some species show only a modest capacity for transcriptional variation. Shifts in microbial densities may offer a primary mechanism to vary expression levels of symbiont functions.

In an ant–bacterial mutualism, the eusocial nature of the host may enhance symbiont plasticity, because the bacteria function within physiologically distinct castes and mediate exchanges among them. In the Blochmannia–Camponotus symbiosis, observed symbiont plasticity is best understood by considering the host colony as a superorganism, with the bacteria meeting the needs of the colony but not necessarily the individual ants that house them. For example, the symbiont is not essential to individual workers, but rather enhances this caste's contribution to colony growth.

Though exceptional in their constrained lifestyle, stable mutualists of insects illustrate general connections among symbiotic lifestyle, genome change, and mechanisms of plasticity. Challenges for future work will include distinguishing adaptive responses from neutral or even harmful perturbations in symbiont functions, and clarifying the actual signals that hosts and symbionts exchange to coordinate their responses.

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APPENDIX: REGULATION GENES RETAINED IN SELECT INSECT MUTUALISTS

Gene	*Escherichia coli* bnumber	*Blochmannia pennsylvanicus*	*Blochmannia floridanus*	*Wigglesworthia brevipalpis*	*Buchnera–Acyrthosiphon pisum*	*Buchnera–Schizaphis graminum*	*Buchnera–Baizongia pistaciae*	Function
alaS	b2697	Bpen174	Bfl168	WGLp234	BU403	BUsg390	Bbp364	Alanyl-tRNA synthetase; transcriptional repressor
birA	b3973	Bpen190	Bfl184	WGLp513	–	–	–	Bifunctional biotin-[acetyl-CoA-carboxylase] ligase and transcriptional repressor
bolA	b0435	–	–	–	BU473	BUsg457	Bbp418	Transcriptional dual regulator
cgtA	b3183	Bpen098	Bfl095	WGLp475	BU389	BUsg376	Bbp352	GTPase involved in DNA replication and ribosome assembly; transcriptional repressor
clpP	b0437	Bpen253	Bfl246	WGLp155	BU475	BUsg459	Bbp419	Serine protease subunit; posttranscriptional regulator
cpxR	b3912	–	–	WGLp538	–	–	–	Transcriptional regulator in 2-component system
cspC	b1823	Bpen462	Bfl448	–	BU322	–	–	Predicted DNA-binding transcriptional regulator
cspE	b0623	–	–	WGLp177	BU489	BUsg473	Bbp433	Transcription antiterminator and regulator of RNA stability
dksA	b0145	Bpen154	Bfl149	–	BU198	BUsg192	Bbp184	RNA polymerase-binding transcription factor
dnaA	b3702	–	–	–	BU012	BUsg012	Bbp012	Transcriptional dual regulator HU-alpha; (HU-2)
fis	b3261	–	–	–	BU400	BUsg387	–	DNA-binding protein; a trans activator for transcription
flhC	b1891	–	–	WGLp031	–	–	–	Regulator of flagellar biosynthesis; transcription initiation factor
flhD	b1892	–	–	WGLp030	–	–	–	Regulator of flagellar biosynthesis; transcription initiation factor
fliA	b1922	–	–	WGLp199	–	–	–	Regulation of flagellar operons
frr	b0172	Bpen282	Bfl274	WGLp389	BU234	BUsg228	Bbp216	Ribosome releasing factor
greA	b3181	Bpen099	Bfl096	WGLp233	BU384	BUsg371	Bbp347	Transcription elongation factor
hflC	b4175	Bpen085	Bfl082	WGLp186	BU567	BUsg547	Bbp512	Regulator of FtsH protease
hfq	b4172	Bpen082	–	WGLp184	–	–	–	RNA-binding protein that affects many cellular processes
hha	b0460	–	–	WGLp524	–	–	–	Hemolysin expression modulating protein
hns	b1237	Bpen448	–	WGLp367	BU272	–	–	H-NS transcriptional dual regulator

(continued)

APPENDIX (CONTINUED)

Gene	Escherichia coli bnumber	Blochmannia pennsylvanicus	Blochmannia floridanus	Wigglesworthia brevipalpis	Buchnera–Acyrthosiphon pisum	Buchnera–Schizaphis graminum	Buchnera–Baizongia pistaciae	Function
hslK	b4174	Bpen084	Bfl081	WGLp185	BU568	BUsg548	Bbp513	Regulator of FtsH protease
hslU	b3931	–	–	WGLp277	BU579	BUsg558	Bbp524	ATPase component of the HslVU protease
hslV	b3932	–	–	WGLp278	BU578	BUsg557	Bbp523	Peptidase component of the HslVU protease
hslX	b4173	Bpen083	Bfl080	–	–	–	–	Putative GTPase; possible regulator of HflKC
hupA	b4000	–	–	WGLp509	BU032	BUsg033	Bbp033	Transcriptional dual regulator HU-α (HU-2)
ihfA (himA)	b1712	–	–	–	BU131	BUsg123	–	Integration host factor alpha
ihfB (himD)	b0912	–	–	–	BU308	BUsg298	–	Integration host factor beta
ispH	b0029	Bpen124	–	–	–	–	–	Control of stringent response
lon	b0439	Bpen307	Bfl299	WGLp153	BU477	BUsg461	Bbp421	ATP-dependent protease
metK	b2942	Bpen259	Bfl252	WGLp316	BU408	BUsg393	–	MetK S-adenosylmethionine synthetase monomer
metR	b3828	–	–	–	–	BUsg030	–	MetR transcriptional dual regulator
mntR	b0817	Bpen403	–	–	–	–	–	Putative toxin
nusA	b3169	Bpen107	Bfl103	WGLp227	BU378	BUsg366	Bbp341	Transcription termination/antitermination L factor
ptsG	b1101	–	–	–	BU356	BUsg344	Bbp326	PTS system, glucose-specific IIBC component
putA	b1014	–	–	WGLp434	–	–	–	Proline dehydrogenase, P5C dehydrogenase
pyrI	b4244	–	–	WGLp590	BU370	BUsg358	–	Aspartate carbamoyltransferase, regulatory subunit
recA	b2699	–	–	WGLp235	–	–	–	DNA strand exchange and renaturation, DNA-dependent ATPase, DNA- and ATP-dependent coprotease
rho	b3783	Bpen607	Bfl586	WGLp303	BU596	BUsg572	Bbp538	Transcription termination factor Rho monomer; polarity suppressor
rplA	b3984	Bpen580	Bfl560	WGLp519	BU037	BUsg038	Bbp038	50S ribosomal subunit protein L1; posttranscriptional regulation of ribosomal protein genes
rplD	b3319	Bpen199	Bfl192	WGLp544	BU523	BUsg504	Bbp466	50S ribosomal subunit protein L4; transcriptional and posttranscriptional regulation of ribosomal protein genes

APPENDIX (CONTINUED)

Gene	*Escherichia coli* bnumber	*Blochmannia pennsylvanicus*	*Blochmannia floridanus*	*Wigglesworthia brevipalpis*	*Buchnera–Acyrthosiphon pisum*	*Buchnera–Schizaphis graminum*	*Buchnera–Baizongia pistaciae*	Function
rplJ	b3985	Bpen579	Bfl559	WGLp520	BU036	BUsg037	Bbp037	50S ribosomal subunit protein L10; posttranscriptional regulation of ribosomal protein genes
rplT	b1716	Bpen365	Bfl354	WGLp084	BU128	BUsg120	Bbp122	50S ribosomal subunit protein L20; autoregulates its own expression and that of L35
rpmH	b3703	Bpen014	Bfl015	WGLp014	BU013	BUsg013	Bbp013	50S ribosomal subunit protein L34; inhibitor of the decarboxylases involved in polyamine biosynthesis
rpoD	b3067	Bpen057	Bfl056	WGLp468	BU055	BUsg052	Bbp052	RNA polymerase, sigmaD factor; primary sigma factor during exponential growth
rpoH	b3461	Bpen653	Bfl626	WGLp072	BU025	BUsg026	Bbp027	RNA polymerase, sigma(32) factor; regulation of proteins induced at high temperatures
rpsH	b3306	Bpen212	Bfl205	WGLp557	BU510	BUsg491	Bbp453	30S ribosomal subunit protein S8; posttranscriptional regulation of ribosomal protein genes
rpsJ	b3321	Bpen197	Bfl190	WGLp542	BU525	BUsg506	Bbp468	30S ribosomal subunit protein S10; role in transcription antitermination
rpsT	b0023	Bpen120	Bfl116	WGLp296	BU151	BUsg144	Bbp140	30S ribosomal subunit protein S20; inhibitor of the decarboxylases involved in polyamine biosynthesis
rseP (yaeL)	b0176	Bpen286	Bfl278	WGLp385	–	–	–	Cleaves RseA, thereby activating sigmaE-mediated stress response
slyA	b1642	Bpen380	Bfl369	WGLp336	–	–	–	Transcriptional regulator for cryptic hemolysin
sufB (ynhE)	b1683	Bpen370	Bfl359	WGLp360	–	–	–	SufBCD complex activates the cysteine desulfurase; secondary pathway of iron-sulfur cluster assembly
sufC (ynhD)	b1682	Bpen371	Bfl360	WGLp359	–	–	–	Component of SufBCD complex
sufD (ynhC)	b1681	Bpen372	Bfl361	WGLp358	–	–	–	Component of SufBCD complex
sufE	b1679	Bpen374	Bfl363	WGLp356	–	–	–	Sulfur acceptor that activates SufS cysteine desulfurase
suhB	b2533	Bpen555	Bfl535	WGLp285	BU285	BUsg274	Bbp264	Enhances synthesis of sigma32 in mutant; extragenic suppressor
thrS	b1719	Bpen362	Bfl351	WGLp081	BU125	BUsg117	Bbp119	Threonyl-tRNA synthetase; translation attenuation and efficiency
uspA	b3495	–	–	–	–	–	Bbp530	Universal stress global stress response regulator
ychA	b1214	–	–	–	BU173	BUsg167	Bbp163	Predicted transcriptional regulator
ychF	b1203	Bpen354	Bfl344	WGLp351	BU191	BUsg185	Bbp180	Putative GTP-binding protein; translation attenuation and efficiency
ydgL (rsxA)	b1627	–	–	–	BU113	–	Bbp108	Integral membrane protein of SoxR-reducing complex; posttranscriptional regulation

(continued)

Appendix (Continued)

Gene	*Escherichia coli* bnumber	*Blochmannia pennsylvanicus*	*Blochmannia floridanus*	*Wigglesworthia brevipalpis*	*Buchnera–Acyrthosiphon pisum*	*Buchnera–Schizaphis graminum*	*Buchnera–Baizongia pistaciae*	Function
ydgM (rsxB)	b1628	–	–	–	BU114	BUsg106	Bbp109	Member of SoxR-reducing complex; posttranscriptional regulation
ydgN (rsxC)	b1629	–	–	–	BU115	–	Bbp110	Member of SoxR-reducing complex; posttranscriptional regulation
ydgO (rsxD)	b1630	–	–	–	BU116	BUsg108	Bbp111	Integral membrane protein of SoxR-reducing complex; posttranscriptional regulation
ydgP (rsxG)	b1631	–	–	–	BU117	BUsg109	Bbp112	Member of SoxR-reducing complex; posttranscriptional regulation
ydgQ (rsxE)	b1632	–	–	–	BU118	BUsg110	Bbp113	Integral membrane protein of SoxR-reducing complex; posttranscriptional regulation
yhdO	b3244	Bpen305	Bfl297	–	BU398	BUsg385	Bbp361	Protease involved in Microcin maturation and sensitivity to the DNA gyrase inhibitor
yidZ	b3711	Bpen038	Bfl038	–	–	–	–	Putative transcriptional regulator LysR-type
yjeA	b4155	–	–	–	BU582	–	Bbp528	Putative regulator of pyruvate oxidase
yqeI	b2847	Bpen401	Bfl390	WGLp467	–	–	–	Predicted transcriptional regulator
yrbA	b3190	Bpen046	Bfl045	WGLp328	BU385	BUsg372	Bbp348	Predicted DNA-binding transcriptional regulator
zur	b4046	Bpen026	Bfl026	–	–	–	–	Putative transcriptional repressor
443 regulation genes		absent from the six mutualists above						

These gamma-Proteobacterial endosymbionts are closely related to *E. coli*, and their gene contents are generally a subset of the *E. coli* genome. To avoid inconsistencies in annotation methods, we first identified *E. coli* orthologs in each mutualist using the reciprocal smallest distance algorithm (Wall *et al.*, 2003). M. H. Serres kindly provided a list of *E. coli* genes that have been assigned to Regulation category in the MultiFun catalog of roles (Serres *et al.*, 2004). In addition to the genes listed above, *E. coli* possesses numerous (443) regulation genes that are absent from the six mutualists considered here. Reflecting their multiple functions in the cell, many genes (including the loci listed here) have more than one functional designation within Multifun. The table lists the primary annotation ID for the orthologs in mutualists.