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# Strategies of genomic integration within insect-bacterial mutualisms

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## Abstract

Insects, the most diverse group of macroorganisms with 900,000 known species, have been a rich playground for the evolution of symbiotic associations. Symbionts of this enormous animal group include a range of microbial partners. Insects are prone to establishing relationships with intracellular bacteria, which include the most intimate, highly integrated mutualisms known in the biological world. In recent years, an explosion of genomic studies has offered new insights into the molecular, functional, and evolutionary consequences of these insect-bacterial partnerships. In this review, I highlight some insights from genome sequences of bacterial endosymbionts and select insect hosts. Notably, comparisons of facultative versus obligate bacterial mutualists have revealed distinct genome features representing different stages along a shared trajectory of genome reduction. Bacteria associated with the cedar aphid offer a snapshot of a transition from facultative to obligate mutualism, illustrating the genomic basis of this key step along the symbiotic spectrum. In addition, genomes of stable, dual bacterial symbionts reflect independent instances of astonishing metabolic integration. In these systems, synthesis of key nutrients, and perhaps basic cellular processes, require collaboration among co-residing bacteria and their insect host. These findings provide a launching point for a new era of genomic explorations of bacterial-animal symbioses. Future studies promise to reveal symbiotic strategies across a broad ecological and phylogenetic range, to clarify key transitions along a spectrum of interaction types, and to fuel new experimental approaches to dissect the mechanistic basis of intimate host-symbiont associations.

## Introduction: Symbiosis at the intersection of genomics and microbiology

Forecasts that the 21st century will mark the “Age of Biology” were well founded (Cracraft, 2004). During the past decade, rapid advances in the life sciences have included two interconnected revolutions. First, genomics and other “-omic” approaches have changed the landscape of our field, allowing biologists to gather genetic data in a high-throughput fashion and to examine patterns and processes at a whole genome scales. These technological advances have spawned an intellectual revolution by changing the very questions we can ask about human health, cell biology, evolution, environmental sciences, and beyond.

Genomic capabilities accelerated yet another fundamental shift: a broad recognition of the fact that we live in a microbial world. While the ubiquity and profound significance of bacteria and other microbes have been understood for decades in certain subdisciplines (e.g., soil sciences, geochemistry, marine biology), genomic datasets and deep community sampling have sparked an appreciation and integration of microbial biology across fields. Biologists as a whole now appreciate the astonishing diversity and central importance of microbes in the evolutionary history and current survival of life.

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At the intersection of these two revolutions lies the burgeoning field of microbial genomics. Exponential increases in sequencing throughput have generated an enormous database for genome comparisons. Building on genomic technology, new abilities to study transcriptomes and proteomes have illuminated bacterial functions in both lab and natural settings, and in both model and non-model, even uncultivable microbial species. The resulting datasets illustrate that the huge genomic and functional diversity among microbes has been shaped by the various environments and species interactions in which these lineages evolved and exist today.

Symbiotic associations between microbes and animals represent some of the most striking examples of how biotic interactions can impact microbial genomes. For example, associations with animals are known to influence microbial genome size, functional capabilities, rates of gene transfer, persistence of parasitic DNA elements, and patterns of nucleotide and protein evolution (e.g., Smillie *et al.,* 2011, McCutcheon and Moran, 2011, Moran *et al.*, 2008, Newton and Bordenstein, 2011). In fact, the wide spectrum of genome diversity documented among bacterial species is largely due to the various impacts of host associations.

The most profound impacts of symbiosis are arguably found in the genomes of obligate bacterial endosymbionts of insects. This is not entirely surprising, given the reliance of these bacteria upon their insect hosts and frequent long-term coevolution with their respective host group (Baumann *et al.,* 2000). Several reviews highlight the profound genome reduction and apparent genome deterioration experienced by many insect endosymbionts, which include the smallest known bacterial genomes (Gil *et al.,* 2004, Moran *et al.,* 2008, Moya *et al.,* 2008, Oliver *et al.,* 2010). I refer the reader to those reviews for a broader overview of insect endosymbiont genomes.

In this review, I focus recent genomic studies that highlight recent transitions along the symbiotic spectrum toward increased metabolic integration, as well as studies of long-term endosymbionts that show a level of intertwining that is far more intimate than anticipated just a few years ago. I also highlight the utility of insect host genomes that, although relatively limited in number, hint at potential impacts of persistent microbial partners on animal lineages.

## Lifestyle features of bacterial mutualists in insects

Among animals, insects are especially prone to establishing symbiotic relationships with intracellular bacteria (Buchner, 1965). Their intimate bacterial partners include reproductive parasites, transient facultative mutualists, and persistent, obligate mutualists. These symbioses offer opportunities to explore the evolution of diverse lifestyles and interaction types. Two such lifestyles are detailed below, as context for interpreting patterns of genomic variation.

First, long-term obligate mutualists in insects are among the most highly constrained, stable, and specialized symbioses known in the animal world. These so-called ‘primary’ bacterial endosymbionts are closely integrated with their insect hosts, live within specialized host cells (called bacteriocytes), and occur in an estimated ~10–20% of insect species (Douglas 1989). These symbioses are typically nutritional in nature, as the bacteria often supplement specific nutrients that are missing in the host’s diet. Not surprisingly, these mutualisms are most common in host groups that feed on unbalanced diets, such as plant sap or vertebrate blood (Buchner, 1965). Symbionts may also perform more general functions such as nitrogen recycling, as shown in ants (Gil *et al.,* 2003, Feldhaar *et al.,* 2007) and cockroaches (Sabree *et al.,* 2009). By virtue of their bacterial associates, insect hosts can thrive on otherwise inadequate diets (e.g., Akman-Gündüz and Douglas, 2009, and reviewed by

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Feldhaar 2011). Their transmission to host offspring leaves nothing to chance. The symbionts are transmitted maternally, typically via the egg. Strict host-symbiont cospeciation (Figure 1a) indicates a high fidelity of this maternal transmission, often for hundreds of millions of years since the symbiosis was established (Baumann *et al.*, 2000). Interestingly, intracellular mutualisms are virtually unknown among vertebrates, an animal group in which intracellular microbes are almost exclusively pathogenic. The sole exception is a beneficial alga that lives within cells of salamander embryos (Kerney *et al.,* 2011).

Among insects, the same species that house obligate intracellular mutualists sometimes possess facultative bacterial mutualists as well (reviewed by Oliver *et al.*, 2010, Moran *et al.*, 2008). These 'secondary' endosymbionts also rely on insect hosts, but are facultative from the host's vantage point. Unlikely primary mutualists, they may be transient, often infect only a fraction of a given host population, and experience a combination of vertical and occasional horizontal transmission across host populations or species (Figure 1b). Facultative mutualists confer environment-specific advantages, such as protection against parasitism by natural enemies, enhancement of host nutrition, or tolerance to thermal stress (Moran *et al.*, 2005b, and reviewed in Oliver *et al.*, 2010). These associates are best studied in aphids, but also occur in a range of insect groups including psyllids, whiteflies, scale insects, tsetse flies, and perhaps ants, which may associate with bacteria related to a secondary symbiont in aphids, *Serratia symbiotica* (He *et al.*, 2011, Sirvio and Pamilo, 2010).

As detailed below, recently sequenced genomes include obligate bacterial mutualists, facultative associates, and the insect hosts themselves. These data have offered insights into the genomic consequences of intimate bacterial associations, mechanisms of metabolic integration, and evolutionary processes that underlie key symbiotic transitions.

## Convergent genome degradation in obligate mutualists

The first insights into genomics of insect endosymbionts occurred with the publication of the full genome sequence of the primary endosymbiont (*Buchnera*) associated with the pea aphid (*Acyrthosiphon pisum*) (Shigenobu *et al.*, 2000). This landmark study revealed massive gene loss across most functional categories as part of a broader picture of severe genome reduction. Lost genes include many cell membrane proteins, DNA repair and recombination genes, repeated and parasitic DNA, and biosynthetic pathways for nutrients that the aphid host can obtain from its diet or synthesize itself (such as non-essential amino acids). Against this background of gene loss, *Buchnera* retains pathways to synthesize key nutrients, such as essential amino acids, that are required by the sap-feeding aphid host. In this sense, the bacterial genome points to a "complementarity and syntrophy between the host and the symbiont" (Shigenobu *et al.*, 2000). Rather surprising at that time, the small *Buchnera* genome shows no distinct "symbiotic genes" (e.g., analogous to nodulation genes in rhizobia), nor any evidence for gene acquisitions via horizontal transfer thought to typify bacterial genome dynamics. Rather, we learned this mutualist is a much-streamlined version of its enterobacterial ancestors, retaining functions required to perform basic cellular processes and to fulfill its symbiotic role.

Since that first snapshot, the exponential increase in bacterial genome sequences has included many insect endosymbionts. These endosymbiont genomes show some uncanny similarities to the trends first observed in *Buchnera*. For instance, primary endosymbionts of insects show a consistent trajectory of genome reduction to sizes <800 kb, and often far smaller (reviewed by McCutcheon and Moran, 2011) (Table 1). In fact, insect endosymbionts include smallest cellular genomes currently known. Specific genes and pathways that are deleted versus kept vary among endosymbiont lineages, depending on

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distinct selective pressures within insect hosts and historical contingencies in the loss of alternative pathways (Pal *et al.*, 2006). However, broadly speaking, mutualist genomes preferentially retain fundamental cellular processes (e.g., transcription, translation) and biosynthesis of nutrients that are missing from the host's diet (e.g., amino acids among sap-feeding insects, or cofactors among blood-feeders). In this sense, genome reduction in primary mutualists consistently reflects functional integration and shared metabolism with the insect host (and detailed below, sometimes with another co-residing bacterial mutualist). Small mutualist genomes also tend to lose redundant pathways, or duplicate components that can perform the same function to a certain extent (Mendonca *et al.*, 2011). This loss of redundancy may reflect relaxed selection for robustness in a predictable, intracellular environment.

With a couple of notable exceptions (highlighted in McCutcheon and Moran, 2011), genomes of primary endosymbionts are extremely AT-rich. Because these bacteria lack many DNA repair functions, their genomes have greater exposure to the universal GC -> AT biased mutation in bacteria, which reflects high rates of C/G to T/A transitions (Hershberg and Petrov, 2010). In addition, obligate mutualists of insects have low recombination (often complete asexuality), likely due to the loss of recombination genes including the striking loss of *recA* in some genomes, a lack of mobile DNA and repeated elements, and reduced opportunities for gene exchange within host cells (Mira *et al.*, 2002).

Such constraints on gene exchange in obligate mutualists limit opportunities to acquire new functions or to replace lost genes via horizontal gene transfer. Unlike most bacteria that experience frequent gene acquisition from foreign sources, apparently these mutualists are constrained to a one-way road of gene loss and, therefore, must live with the functions they have. In the absence of gene acquisition, once a function is lost, apparently it is forever lost in that particular genome. This irreversible gene loss may constrain the evolutionary potential of the symbiont and host alike. Genome sequences have shown the surprising absence of nutritional functions in certain mutualist lineages, such as cysteine biosynthesis (*cys*) genes in *Buchnera* of the green bug aphid *Schizaphis graminum* (Tamas *et al.*, 2002), and the loss of glutamine synthetase (*glnA*) in *Blochmannia* of the ant *Camponotus vafer* (Williams and Wernegreen, 2010). Moreover, a substantial loss of regulation genes explains why mutualists exhibit only modest shifts in transcript abundances in response to heat shock (Wilcox *et al.*, 2003), distinct diet treatments (Moran *et al.*, 2005a) and host developmental stages (Stoll *et al.*, 2009).

Primary mutualists also show convergent patterns at the protein and DNA sequence level. They have rapid evolutionary rates, unusually low structural stability of 16S rRNA, and an excess of nonsynonymous substitutions in proteins (Moran, 1996). Their proteins tend to accumulate destabilizing amino acid substitutions that likely reduce the efficiency of protein folding and may lead to misfolding and aggregation (Van Ham *et al.*, 2003). Many such mutualists constitutively over-express the chaperonin GroEL (Ishikawa, 1984), perhaps as a compensatory mechanism to cope with degraded protein structures (Moran, 1996).

This convergent trajectory of genome evolution across primary insect mutualists is stunning. Like most trends in nature, their shared features are best understood in an evolutionary framework. Their genomic similarities likely reflect parallel shifts in fundamental evolutionary processes that are predictably associated with transitions to obligate endosymbiosis. For example, living exclusively within insect cells must involve significant changes in selective regime. Relaxed selection on many metabolic functions no longer required in a host cellular environment probably explains a good fraction of gene deletions, while host-level selection retains key nutrient biosynthesis pathways. In addition, obligate host-dependence is coupled with a significant reduction in $N_e$ due to maternal transmission

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and recurrent population bottlenecks (Abbot and Moran, 2002, Andersson and Kurland, 1998, Funk *et al.*, 2001, Mira and Moran, 2002), which is expected to increase impacts of genetic drift and to accelerate the fixation of slightly deleterious mutations in proteins (Moran, 1996). Strong effects of drift might contribute to deleterious gene deletions, perhaps explaining the early loss presumably beneficial functions (e.g. DNA repair) and some surprising losses of biosynthetic genes (as noted above). In the absence of frequent recombination with genetically distinct strains, these symbiont genomes are not a hospitable environment for phage, transposable elements, insertion sequences, and other mobile DNA. The loss of these elements may further constrain gene exchange, exacerbating effects of genetic drift under reduced $N_e$. In short, shared aspects of a symbiotic lifestyle contribute to the striking convergence in primary endosymbiont genomes.

## Facultative mutualists offer snapshots of early genome flux

Although ancient events are difficult to reconstruct, phylogenetic evidence suggests that some primary mutualists may have arisen from facultative endosymbionts in insects (Husnik *et al.*, 2011, Wernegreen *et al.*, 2009). In this case, facultative and obligate associates may represent points along a continuous spectrum of symbiosis. As younger endosymbionts, facultative mutualists provide an earlier snapshot of genomic changes that occur when bacteria adopt a host-associated existence (Moran and Plague, 2004).

Supporting this view, the highly dynamic features of facultative endosymbiont genomes have been linked to their relatively recent host-dependence. Data for the tsetse fly secondary endosymbiont *Sodalis glossinidius* (Toh *et al.*, 2006), and the aphid secondary endosymbionts Serratia symbiotica (Burke and Moran, 2011), Regiella insecticola (Degnan et al., 2010), and *Hamiltonella defensa* (Degnan *et al.*, 2009) point to genome turmoil within these relatively young host-dependent lineages. Their genomes are characterized by prevalent gene inactivations leading to abundant pseudogenes, an early stage in genome reduction. These pseudogenes are expected to shrink over time due to an underlying mutational bias in bacteria (Mira *et al.*, 2001).

Facultative mutualists also show a high density of insertion sequences and other mobile elements, perhaps reflecting a vulnerability to parasitic DNA in species that underwent a recent reduction in $N_e$ but still experience gene exchange (reviewed by Moran and Plague, 2004). The eventual loss of these elements in primary mutualists may be explained by their switch to strict asexuality (e.g., due to strict clonality within host cells and/or loss of recombination genes), making their genomes less hospitable for DNA elements that require horizontal transmission. In facultative mutualists, recombination among insertion sequences and other repeated elements catalyze large inversion and deletions. Although recombination is diminished or lost in primary mutualists, comparisons of gene order suggest an historic period of large-scale rearrangements. In this sense, the distinct genome contents, architecture, and dynamics of facultative and obligate insect mutualists may be explained by their status as recent versus ancient stages along a shared evolutionary trajectory.

## Recent insights: Transitions from facultative to obligate mutualism

The affiliation between facultative and primary mutualists is also apparent in their functions. Experimental work suggests these bacteria can perform similar functions for insect hosts, at least in some circumstances. In controlled settings, facultative endosymbionts can compensate for the loss the primary symbiont and ultimately replace them. Specially, Koga *et al.* (2003) found that the presence of a facultative mutualist, *Serratia*, permitted the survival and reproduction of aphids from which *Buchnera* had been eliminated. This secondary symbiont also infected the bacteriocytes that otherwise contain *Buchnera*, essentially usurping the symbiotic niche both physiologically and cytologically. The authors

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suggest that *Serratia* may supply, perhaps less efficiently, essential amino acids and other nutrients that are typically provisioned by *Buchnera*. Combined with phylogenetic data supporting close relationships between primary and secondary symbionts, functional compensation raises the possibility that, over evolutionary time, secondary symbionts might occasionally form a dual symbiosis with an existing primary mutualist (Figure 1c), or even replace older symbiont lineages (Figure 1d).

Evolutionary studies of weevils provide evidence that replacements of primary mutualists by other bacterial associates have indeed occurred historically. In the Dryophthorid weevil family, the established, primary mutualist *Nardonella* has been replaced by different endosymbiotic bacteria, such as the *Sodalis*-like endosymbiont in *Sitophilus* (grain weevils) (Heddi *et al.*, 1998), and as-yet unnamed gamma Proteobacteria lineages in two other weevil genera (Lefevre *et al.*, 2004). While it remains uncertain whether these patterns reflect replacement of *Nardonella* by a former secondary endosymbiont per se, a recent study found that *Sodalis*-like secondary symbionts occur in weevils and might provide a source for symbiont replacements (Toju *et al.*, 2010).

## A genomic ‘smoking gun’ captures a facultative to obligate transition

The transition of a facultative mutualist into an obligate one has been rather speculative until recently. As detailed below, the genome sequences of two unusual endosymbionts from the cedar aphid (*Cinara cedri*) combined with earlier experimental work provide the ‘smoking gun’ of a recent transition from facultative to obligate mutualism in nature. In this aphid species, a formerly secondary endosymbiont has teamed up with the primary mutualist, *Buchnera*, to form a highly integrated, obligate mutualism (Lamelas *et al.*, 2011, Perez-Brocal *et al.*, 2006).

A few years ago, we learned that *Buchnera* associated with the cedar aphid has undergone a shocking level of genome reduction (Perez-Brocal *et al.*, 2006) (Table 1). At just 416 KB, the smallest *Buchnera* genome yet described, it lacks the ability to synthesize tryptophan and riboflavin. The authors proposed that the mutualistic capabilities of this *Buchnera* strain might be complemented by an abundant *Serratia symbiotica* endosymbiont that not only occurs in the same aphid species, but also (unlike other ‘secondary’ endosymbionts) was shown to be required for host survival. Division of labor in the synthesis of tryptophan may fuel this pattern. *Buchnera* of *C. cedri* encodes *trpEG* on a plasmid, but surprisingly has lost *trpDCBA* required for tryptophan biosynthesis (Perez-Brocal *et al.*, 2006). Instead, these missing *trp* genes are encoded on the chromosome of the cedar aphid’s *Serratia* associate. Subsequent experimental studies showed that, as predicted, tryptophan biosynthesis is shared between the *Buchnera* and *Serratia* in this host species (Gosalbes *et al.*, 2008).

These results suggested that *Serratia symbiotica* in the cedar aphid is not your typical secondary endosymbiont. Supporting its distinct status, phylogenetic work showed this particular *Serratia* strain belongs to a cluster (cluster B) that is distinct from *Serratia symbiotica* that act as facultative mutualists in pea aphids and other hosts (cluster A) (Burke *et al.*, 2009, Lamelas *et al.*, 2008). Along with its requirement for host survival, the cluster B strain shows other signatures of primary endosymbiosis. Its cells resemble those of *Buchnera* (large, round, and pleomorphic) rather than the typical rod-shaped cells of cluster A *Serratia symbiotica* (Gomez-Valero *et al.*, 2004). Moreover, it is confined to a second type of bacteriocyte that, notably, are as abundant as *Buchnera*-filled bacteriocytes in the cedar aphid host (Gomez-Valero *et al.*, 2004). Although cluster B does not show patterns of cospeciation with its hosts (Burke *et al.*, 2009), the cumulative data suggested this strain recently transitioned to obligate mutualism.

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Genomic data provided the smoking gun that *S. symbiotica* in *C. cedri* (or, *S. symbiotica* SCc) is a 'missing link' in the transition from facultative to obligate mutualism. In contrast to the genome sequence of the related, "cluster A" facultative mutualist from pea aphids (*S. symbiotica* SAp) (Burke and Moran, 2011), the *S. symbiotica* SCc genome shows hallmark features of obligate mutualism: low %GC (29.2% GC, compared to 52% GC for *S. symbiotica* SAp), a single rRNA operon (compared to 5 in *S. symbiotica* SAp), severe reduction in genome size, deletion of the *recA* gene, as well as the lack of ISs or other mobile DNA (Lamelas *et al.*, 2011). While metabolic reconstruction highlight some distinct responsibilities of *S. symbiotica* SCc and *Buchnera* BCc (e.g., the synthesis of cofactors, vitamins and nucleotides by *S. symbiotica*, and synthesis of many amino acids by *Buchnera*), several pathways involve the contribution of both bacterial partners. For instance, metabolic complementation likely occurs the synthesis of biotin, folate, and CoA, and most famously, tryptophan. This symbiosis represents an interconnected, obligate mutualism involving two bacteria and the insect host.

One possible scenario for the establishment of this dual bacterial symbiosis is the loss of key metabolic genes in a secondary endosymbiont and its subsequent reliance on *Buchnera* (Lamelas *et al.*, 2011). Continued gene deletions and functional loss in *Buchnera* may lead to compensation by the co-occurring endosymbiont. Interdependence surrounding the biosynthesis of just one essential nutrient, such as tryptophan, may be enough to "seal" the interaction and initiate a trajectory of increased reliance (Lamelas *et al.*, 2011). Over time, continued functional streamlining could lead to the integrated, dual mutualism observed today.

## Interconnected metabolism in ancient dual mutualisms

Recent work has taught us that highly integrated, dual bacterial mutualisms are not rare exceptions; rather, they have evolved multiple times across insect host lineages. Dual symbioses include very ancient associations whose origins may have resembled the more recent *Serratia symbiotica–Buchnera* partnership in *C. cedri*. In these symbioses, both bacteria are required to provide a full complement of essential nutrients, but the overall trajectories of metabolic integration show variations on a theme. For instance, sometimes the production of broad nutrient categories (e.g., amino acids, cofactors) is distributed between the two co-residing bacterial genomes, but the complete (or near complete) biosynthetic pathway for any given nutrient occurs in just one bacterial species. As an example, the two primary endosymbionts in some sap-feeding insects encode biosynthesis pathways for distinct but complementary amino acids. Combined, the bacteria can synthesize the ten essential amino acids required by the insect host. For example, most essential amino acids are synthesized by the endosymbiont *Sulcia*, and the remainding few are synthesized by coresiding dual endosymbionts: *Baumannia* in sharpshooters (McCutcheon and Moran, 2007), *Zinderia* in spittlebugs (McCutcheon and Moran, 2010), and *Hodgkinia* in cicadas (McCutcheon *et al.*, 2009). In this sense, metabolic integration occurs at the level of broad nutrient categories (here, amino acids).

Recent genome studies illustrate even tighter integration in the metabolism of co-residing endosymbionts. In these cases, biosynthesis of one particular nutrient is shared between the two bacteria. Tryptophan synthesis by *Buchnera* plus *Serratia symbiotica* within the cedar aphid offers one salient example. In addition, a remarkable dual symbiosis in mealybugs consists of a gamma-Proteobacterium (*Moranella*) that lives within the cells of a beta-Proteobacterium (*Tremblaya*), which in turn resides within mealybug bacteriocytes (von Dohlen *et al.*, 2001). Genomic analysis of this association revealed seamless functional integration, in which synthesis of particular key nutrients (e.g., tryptophan and threonine) require enzymes from both bacteria (McCutcheon and von Dohlen, 2011). In addition,

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synthesis of phenylalanine, arginine, and isoleucine require enzymes encoded by both bacteria and perhaps host functions as well. This division of labor probably involves the shuttling of metabolites between the two bacteria and the mealybug host, but the mechanisms of such exchanges are unclear.

For *Tremblaya*, functional integration coincides with unprecedented genome reduction. The smallest known bacterial genome, this species devotes most of its 139-kb genome to translation and amino acid biosynthesis. Even so, its genome is missing functions considered critical, such as aminoacyl-tRNA synthesis and translational release factors. *Moranella* or the mealybug host might supply these proteins. This incredible level of cellular and metabolic integration challenges the distinction between endosymbiont and organelle status (Keeling, 2011).

## Insect host genomes: Signatures of persistent microbial partners

Compared to the abundant genomic data for bacterial endosymbionts, insect host sequences are relatively scarce. Genome sequences are available for a few insect groups that have coevolved with obligate bacterial mutualists: an aphid, louse, and carpenter ant. Genomes in progress include the tsetse fly, whitefly, and psyllid (based on projects listed at http://www.ncbi.nlm.nih.gov/genome/browse/). The availability of host sequences offers new opportunities to assess impacts of long-term mutualisms on animal genomes. Below, I address just a few potential influences: gene transfer, immune functions, and biosynthetic capabilities.

Given the antiquity of many insect-bacterial mutualisms, combined with extreme genome reduction of bacterial partners, we might expect to find transfer of bacterial genes into the insect host genome. Earlier work found frequent gene transfer from the reproductive parasite *Wolbachia* into insect host nuclear genes (Dunning Hotopp *et al.*, 2007, Dunning Hotopp, 2011). However, to date, signatures of functional gene transfer from primary endosymbionts to their hosts' genomes have not been detected. The pea aphid genome contains 12 genes of bacterial origin, several of which are apparently functional and highly upregulated in bacteriocytes; however, those genes transferred from *Buchnera* are not functional (Nikoh *et al.*, 2010). In addition, neither the human body louse genome (Kirkness *et al.*, 2010) nor the carpenter ant genome (Bonasio *et al.*, 2010) showed signs of gene transfer from primary endosymbionts (although the ant genome is not yet closed, so transfer is difficult to rule out). It remains to be seen whether these patterns reflect a consistent lack of functional gene transfer or simply result from the very limited taxonomic sample. Of particular interest will be potential gene transfer when the bacterial genome is exceedingly small, such as *Carsonella* in psyllids.

Expansion of the insect genome database will let us test whether immune functions evolve differently in host groups that constantly interact with essential microbes. We know that successful establishment and control of bacterial symbioses involve close coordination with the host immune response (reviewed in Gross *et al.*, 2009). Recent work shows that the antimicrobial peptide coleoptericin-A (ColA) in weevils controls bacterial mutualists, by specifically inhibiting cell division of bacteria within the host bacteriocytes (Login *et al.*, 2011). In ants, injection of *Blochmannia* into the ant hemocoel triggers a host immune response, suggesting the ant immune system could be involved in controlling symbiont populations (Ratzka *et al.*, 2011). These examples suggest that host immune responses may actively control symbiont numbers.

The impacts of persistent microbial associates on the host innate immune system are largely unclear, but host genome sequences offer data to explore this question. In some instances, immune functions may be constrained in order to accommodate microbial partners. As

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reviewed by Shigenobu and Wilson (2011), the impact of bacterial symbionts on immune gene repertoires has been studied in aphids. Pea aphids lack some genes considered essential for recognizing and killing microbes, and gene expression analysis showed relatively few immune-related products (Gerardo *et al.*, 2010). Genes involved in recognition, signaling, and some antimicrobial peptides are missing from this aphid genome, though genes involved in Toll and other signaling pathways are present. Among the many possible explanations for these functional losses is that long-term maintenance of *Buchnera* might have favored the simplification of the aphid immune system. Consistent with this hypothesis, infection of *Drosophila* cells *with Buchnera* triggers an immune response involving fly genes that are missing in aphids (Douglas *et al.*, 2011). However, other work questions the inference of immune system reduction in aphids (and similar inferences for social insects). An alternative interpretation is that relatively well-sampled dipterans (flies, mosquitoes, etc.) have unusually expansive immune systems, compared to the smaller number of immune genes found in aphids, ants, solitary wasps, and bees (Fischman *et al.*, 2011). Future analyses of gene repertoires will benefit from more complete annotations and broader phylogenetic diversity of available insect genomes.

Insect genomes also illuminate the metabolic integration of hosts and microbial symbionts. For example, genome data have facilitated transcriptomics, proteomics, and other experimental approaches to study host-symbiont metabolic interactions. A recent transcriptome analysis revealed that expression of aphid genes within bacteriocytes is closely tied to symbiont capabilities (Hansen and Moran, 2011). Host genes up-regulated in bacteriocytes included genes for amino acid biosynthetic pathways that compensate for functions lost in *Buchnera*, as well as genes involved in the assimilation of ammonia into glutamate, which serves as nitrogen source in synthesis of other amino acids. Likewise, the aphid genome sequence facilitated a global proteomic analysis, which found that aphid enzymes for amino acid metabolism are enriched in bacteriocytes (Poliakov *et al.*, 2011).

From an evolutionary standpoint, the expanding set of insect genomes will let us test the impacts of bacterial partners on the metabolic capabilities that hosts encode, by comparing these genomes to related insects that lack persistent mutualists. Considering obligate microbial mutualists writ large, we know such associations have left a distinct mark on the insect genomes. Specifically, the farming of fungal gardens, complex consortia that include obligate bacterial and fungal mutualists (Currie, 2001), apparently influenced the genomes of leaf-cutter ants. Both genomes available for leaf-cutter ants (*Atta cephalotes* and *Acromyrmex echinatior*) lack biosynthetic genes for arginine, while the five other available ant genomes possess this pathway (Gadau *et al.*, 2012, Suen *et al.*, 2011, Nygaard *et al.*, 2011). One interpretation is that leaf-cutters obtain sufficient arginine by gardening their cultivated fungus, which serves as their primary food source. We might expect to find similar genomic impacts among insects that have coevolved with intracellular nutritional mutualists.

## Conclusions and Prospects

Mutualisms between bacteria and insects include some of the most intimate symbioses known in the biological world. Genomic data is transforming our understanding of these relationships. To date, genome sequences of numerous bacterial endosymbionts illustrate convergent genome reduction and corresponding metabolic integration. Genomic studies have captured transitions along the symbiotic spectrum from facultative and obligate mutualism, illuminating the evolutionary, functional, and molecular basis of key lifestyle shifts. Recent work has also documented unprecedented levels of genomic integration in co-occurring mutualists of sap-feeding insects, reflecting coevolution of two bacterial partners with each other and with their insect host.

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When considering the genomes of the insect hosts themselves, the influence of persistent microbial mutualisms appear to be more subtle. Such impacts may include changes in selection pressures on repertoires of immune genes and metabolic capabilities. In the coming years, more complete annotations of insect genomes and a broader taxonomic sampling, including close relatives that lack persistent bacterial mutualists, will facilitate comparisons.

In a commentary on new insect genomes, Gadagkar (2011) noted that the publication of a full genome sequence is like a "postcard sent home by a visitor giving first impressions of a city – say, New York – after spending just a day." These first impressions are valuable, but only hint at the complex culture, architecture, economy, politics, and other aspects of city life that further exploration will reveal. Likewise, our first impressions of bacterial-insect symbioses, however remarkable, are just the starting point. Insects represent a rich playground for symbiosis, but only a small percentage of host species have been studied with respect to their bacterial partners, and fewer yet the subject of genomic studies. Building on recent work, genomic approaches promise to shed light on an incredible phylogenetic and ecological diversity of insect-microbe associations, clarify processes and outcomes of genomic integration, and fuel new experimental approaches to study the mechanistic basis of these interactions.

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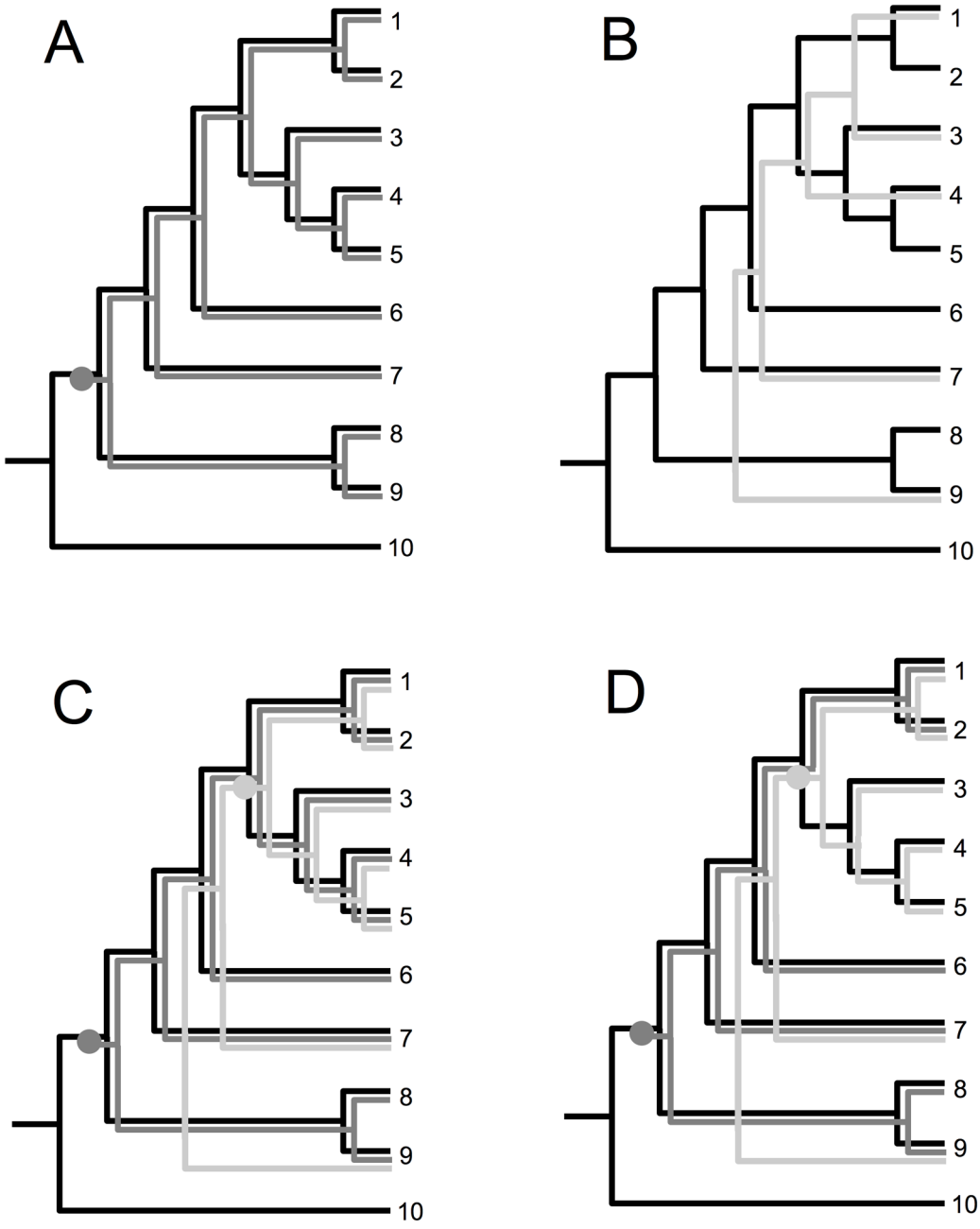


**Figure 1. Evolution of insect-bacterial mutualisms under different scenarios**

In these schematic phylogenies, taxa 1–10 refer to distinct insect species and their associated endosymbionts. Biological examples of each scenario are detailed in the text. **(A) Strict cospeciation**: Cospeciation between insect hosts (black) and primary mutualists (dark grey) generates a classic pattern of identical host and symbiont phylogenies (i.e., phylogenetic congruence). The grey circle marks the lineage along which the symbiosis originated. Cospeciation, typical of the obligate nutritional mutualisms discussed in this review, is often characterized by close host-symbiont genomic integration. **(B) Symbiont loss and host-switching**: By contrast, facultative symbionts (light grey) may be lost along host lineages and experience horizontal transmission among host species. These processes disrupt the congruence between host and symbiont phylogenies. **(C) Teaming up:** In a more complex

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evolutionary scenario, a formerly facultative endosymbiont may join an existing primary mutualist to form a stable, dual mutualism with the host (as illustrated for taxa 1–5). The light grey circle marks the transition from facultative to obligate mutualism for the second bacterial partner. This process might account for the dual bacterial mutualisms in some sap-feeding insects**. (D) Symbiont replacement:** Facultative symbionts may ultimately replace existing primary mutualists in some host lineages, as suggested in weevils. In this schematic example, the acquisition of a second, stable symbiont generated a dual partnership in the lineage leading to taxa 1 and 2, but led to symbiont replacement in taxa 3–5.

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**Table 1**

**Genome sizes of diverse bacterial endosymbionts in insects**

Several groups listed were recently sequenced and are referenced in the text. Species engaging in dual, obligate symbioses are grouped together. Host groups are listed in very general terms. When multiple genomes are available, a range of chromosome size is listed.

| bacteria | bacterial group | insect host groups | dual symbiosis partner | chromosome size (Mb) |
|---|---|---|---|---|
| obligate insect mutualists | | | | |
| *Tremblaya* | beta Proteobacteria | mealybugs | *Moranella* | 0.14 |
| *Moranella* | gamma Proteobacteria | mealybugs | *Tremblaya* | 0.54 |
| *Sulcia* | Bacteroidetes | Auchenorrhyncha (sharpshooters, cicadas, hoppers, and spittlebugs) | *Zinderia, Hodgkinia,* or *Baumannia* | 0.24 – 0.28 |
| *Hodgkinia* | alpha Proteobacteria | cicadas | *Sulcia* | 0.14 |
| *Zinderia* | beta Proteobacteria | spittlebugs | *Sulcia* | 0.21 |
| *Baumannia* | gamma Proteobacteria | sharpshooters | *Sulcia* | 0.69 |
| *Buchnera* BCc | gamma Proteobacteria | cedar aphid (*Cinara cedri*) | *S. symbiotica* SCc | 0.43 |
| *Serratia symbiotica* SCc | gamma Proteobacteria | cedar aphid | *Buchnera* BCc | 1.80 |
| *Carsonella* | gamma Proteobacteria | psyllids | | 0.16 |
| *Riesia* | gamma Proteobacteria | lice | | 0.57 |
| *Blattobacterium* | Flavobacteria | cockroachs, termites | | 0.59 – 0.64 |
| *Buchnera* (of other aphids) | gamma Proteobacteria | aphids | | 0.63 – 0.65 |
| *Wigglesworthia* | gamma Proteobacteria | tsetse flies | | 0.71 |
| *Blochmannia* | gamma Proteobacteria | ants of the tribe Camponotini | | 0.71 – 0.79 |
| facultative insect mutualists | | | | |
| *Serratia symbiotica* SAp | gamma Proteobacteria | pea aphid (*A. pisum*) | | ~2.79 |
| *Sodalis glossinidius* | gamma Proteobacteria | tsetse flies | | 4.17 |
| other bacteria (for reference) | | | | |
| *Mycoplasma genitalium* | Firmicutes | | | 0.58 |
| *Rickettsia prowazekii* | alpha Proteobacteria | | | 1.11 |
| *Escherichia coli* | gamma Proteobacteria | | | 4.64 |