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Genetic Innovations in Animal-Microbe Symbioses

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Abstract

Animal hosts have initiated myriad symbiotic associations with microorganisms, and often have maintained these symbioses for millions of years, spanning drastic changes in ecological conditions and lifestyles. The establishment and persistence of these relationships requires genetic innovations on the parts of both symbionts and hosts. The nature of symbiont innovations depends on their genetic population structure, categorized here as open, closed or mixed. These categories reflect modes of inter-host transmission that result in distinct genomic features, or genomic syndromes, in symbionts. Although less studied, hosts also innovate in order to preserve and control symbiotic partnerships. New capabilities to sequence host-associated microbial communities and to experimentally manipulate both hosts and symbionts are providing unprecedented insights into how genetic innovations arise under different symbiont population structures, and how these innovations function to support symbiotic relationships.

Toc Blurb

The evolutionary persistence of animal symbioses depends on both host and symbiont innovations. Perreau and Moran review how genome sequencing and related experiments have clarified how these innovations arise under different symbiont population structures, categorized here as open, closed and mixed.

Introduction

Symbiotic associations with microbes have shaped animal evolution and contributed to the immense diversity in development, morphology, and lifestyles seen across animal phyla[1]. Many of these symbioses are ancient, dating to the origin of major animal clades, and have had to adapt to shifts in dietary resources, the emergence of new pathogens, and other changing selective pressures.

Appreciation of the dominant role of symbiosis in animal biology and human health has been relatively recent, spurred by the introduction of affordable sequencing methods about

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Author contributions

The authors contributed equally to all aspects of the article.

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[Au: thank you for including key points. These have been moved to a separate document.]

15 years ago. Since then, genomic and metagenomic sequencing of hosts and symbionts has given a picture of capacities, variability, and evolution of symbiotic systems[2–8]. More recently, genetic tools that enable validation of genes and pathways underlying specific symbiont functions have been developed, despite challenges of culturing and experimentally manipulating symbiotic organisms[9,10]. As illustrated in this Review, these approaches have revealed a number of surprising mechanisms through which beneficial symbioses have been successfully maintained over long periods, or have completely transformed themselves, through changes in symbionts and in hosts. These mechanisms can seem bewilderingly diverse, as symbioses evolve through different routes. We argue that this variation is more comprehensible by recognizing that it is largely dictated by the genetic population structure imposed on the symbionts (Figure 1).

The rapid expansion in complete sequences of bacterial genomes has revealed distinct sets of correlated genomic characteristics that arise from differences in evolutionary forces acting on particular lineages[11]. Here, we define three categories of ‘genomic syndromes’ in bacterial symbionts that correspond to different modes of symbiont evolution. We refer to these as ‘open’, ‘closed’, and ‘mixed’ symbioses. Distinguishing these categories allows us to appreciate why symbiotic relationships innovate in strikingly different ways (Figure 1). In open symbiotic communities, exemplified by most gut microbiomes, microbes repeatedly colonize hosts from external, environmental niches, and innovation occurs through turnover of lineages or exchange of genetic material within and between microbial species. At the other extreme, in closed symbioses, symbionts are intimately incorporated into host development and reliably maternally transmitted along with the hosts’ own genes. This symbiont transmission mode **[G]** enforces strict clonality, causing genomic erosion where symbiont lineages lose rather than gain genes, and limiting symbiont responses to novel selective pressures[6,12,13]. Instead, innovations in closed symbioses often involve changes in hosts, such as adoption of entirely new microbial partners[14] or acquisition of novel host genes from bacterial sources[15]. Mixed symbiotic systems regularly rely on vertical transmission from mother to progeny, but also undergo occasional horizontal transmission between host individuals or species. These mixed symbiotic systems exhibit features of both open symbioses, such as frequent gene exchange, and of closed symbioses, including extensive gene loss and rapid sequence evolution.

In this Review, we first summarize the features of different genomic syndromes, as revealed by the growing availability of genomic sequences from bacterial symbionts of diverse animal hosts, and we link these to different evolutionary modes associated with population structure. We then highlight recent discoveries that reveal how genetic and functional innovations arise under each mode of symbiosis evolution. We focus on innovations specifically involved in maintaining the symbiosis itself, while noting that symbiosis can launch hosts into novel niches and lifestyles, resulting in further adaptations. We also emphasize symbiont innovations, largely because these are currently better studied than those of hosts. We do not review the literature on functions of animal microbiomes, as these topics are covered elsewhere[1]. Likewise, we refer readers to previous reviews for specific aspects of symbiosis, including transmission mechanisms[16,17], genome reduction[2,18], how symbioses evolve[6,12,13,19], and horizontal gene transfer into host genomes[15]. We cite

examples from recent studies on a variety of symbioses, rather than covering particular systems in depth.

Symbiosis and genomic syndromes

The classification of a symbiosis as open, closed or mixed is largely determined by inter-host transmission routes and their consequences for genetic population structure of the symbionts (Figure 1). These different symbioses exhibit some commonalities; for example, all animal symbionts must contend with host immune systems. But the expansion in the set of sequenced symbiont genomes has revealed that their modes of evolution, and their resulting genomic features, differ strikingly. Notably, these categories do not neatly fit with function-based classification; for example, all three types can be involved in nutrition or defense of hosts. Likewise, they do not correspond to locations in the host body or tissues, as all three types can be intra- or extracellular, or gut-associated or bacteriome-associated. Most gut symbioses are open, and many bacteriome symbioses are closed, but exceptions occur in both cases.

Open symbioses.

Open symbioses vary in structure and complexity but share a common feature: symbionts are readily exchanged among host individuals or species, and, in some cases, acquired from non-host environmental niches[20–22]. Crucially, the ability of symbionts to come into contact with conspecific strains or with other bacterial species, either within or outside of their host, allows symbionts to acquire genetic material through genetic recombination **[G]**, either via homologous recombination or horizontal gene transfer (HGT). As a consequence, symbionts in these relationships possess genomes similar to those of widespread environmental bacteria, as reflected in typical genome sizes and gene numbers, typical GC content, high coding density, and strain-specific differences in gene content (Figure 2A)[11]. As for most free-living bacteria, their genomes are under effective purifying selection **[G]** to eliminate deleterious mutations, as indicated by low rates of protein evolution relative to DNA sequence evolution (dN/dS **[G]** values < 0.1) (Figure 2A)[11,21].

Open systems can include many symbiont species, as in human and termite guts, or few species, as in honey bee guts, or even a single species, as for *Aliivibrio fischeri* in light organs of bobtail squid and *Burkholderia insecticola* in midgut crypts of the bean bug[5,23]. Most open symbioses involve extracellular symbionts that are exposed to the outside environment, such as symbionts associated with guts, and with surfaces of corals and sponges[24]. However, some involve intracellular symbionts, including the sulfur-oxidising and the methane-oxidising bacteria that live as multiple strains within bacteriocytes **[G]** of *Bathymodiolus* deep sea mussels[25,26].

Closed symbioses.

In closed symbioses, symbiont lineages are clonal, often due to strict maternal transmission. Clonality and population bottlenecks impose small effective population sizes and genetic drift, which leads to degradation and diminution of genomes and loss of functions; these features have been documented repeatedly through genome sequencing of symbiotic

bacteria in insects and other invertebrates[6,18,27] (Figure 2B). Closed symbioses are often millions of years old, as evidenced for symbioses of many insects[27] and of gutless marine flatworms[28] by matching molecular phylogenetic trees of symbionts and hosts calibrated for host lineage age using fossil evidence (Figure 3A). Commonly, such symbionts provide crucial services to hosts, such as provisioning of essential amino acids and vitamins. As a consequence, these are usually mutually obligate associations, required for host development and reproduction. In long-established closed symbioses, symbionts are effectively fused with hosts, approaching the status of organelles[13]. Prime examples include bacterial clades that are restricted to living only in a given group of insect hosts: *Buchnera aphidicola* in aphids, *Blochmannia* spp. in carpenter ants, *Blattabacterium* spp. in cockroaches, and *Sulcia muelleri* in leafhoppers and related insects[27]. But strict uniparental transmission is not required; closed symbioses include any cases where exclusive colonization by a single symbiont strain eliminates opportunity for inter-strain recombination. Thus, recent analyses of genome sequences of light organ symbionts of anglerfish show that they occupy host organs as single clones, and exhibit genome reduction, even though they are acquired environmentally and do not show codiversification with host matrilines[29]. In many closed symbioses, bacteria live within specialized host cells (for example,[30]), but they may be extracellular, as in the pectinase-producing *Stammera* symbiont of tortoise beetles[31], and midgut crypt symbionts of urostylidid, parastrachiid, and plataspid stinkbugs[32–34]. Although closed symbiotic systems have been documented most extensively for insect hosts[27], parallel cases are known from other groups including anglerfish[29], tunicates[35], clams[36], marine flatworms[8], and protists[13,37].

Mixed symbioses.

Some symbioses involve host-restricted bacteria that are routinely transmitted maternally but that occasionally jump between host matrilines within, and sometimes between, species. Examples of mixed systems include *Wolbachia* spp. in arthropods and *Hamiltonella defensa* in aphids; these are predominantly transmitted through direct infection of progeny within the mother, but phylogenetic analyses show that they occasionally undergo horizontal transfer to novel hosts[38,39]. Mixed symbioses share features with both open and closed symbioses, depending on their potential for recombination. Symbiont genomes may recombine and acquire genes within co-infected hosts; but they undergo loss of ancestral genes, genome shrinkage, and accelerated sequence evolution as a result of clonality and genetic drift. Rates of mutation and genome rearrangement can be extremely high; for example, experimental evolution studies revealed that *Spiroplasma* symbionts within laboratory stocks of *Drosophila* spp. undergo rapid changes as evident both from genomic sequencing and observation of symbiont-based host phenotypes[40]. Outbreaks of transposable elements, large deletions, and rearrangements are typical in symbiont genomes of mixed systems (Figure 2C)[41]; these are largely absent from genomes of closed symbioses, which lack mobile elements and exhibit gene order conservation[42,43]. Horizontal transmission, even if infrequent, erases signatures of co-cladogenesis with hosts (Figure 3B) and generates occasional co-infections, thereby creating arenas for genetic exchange and acquisition of novel genes, via bacteriophage or other mobile units[44]. Genomic signatures of mixed systems depend both on frequency of horizontal transmission and the age of the symbiosis[2,6,17,36,45]. Symbionts in mixed systems can be deleterious and/or beneficial to

hosts. For example, *Wolbachia* often is a reproductive parasite that lowers fitness of male hosts but also protects hosts against pathogens or contributes to nutrition[46–48].

Insights into symbiont evolutionary routes from genomic sequencing.

The onslaught of genomic sequencing of symbionts is the main basis for recognizing these different symbiotic categories, as the same genomic syndromes have emerged across bacterial phyla and across various animal hosts. For example, symbiont genomic features, such as large size and evidence of ongoing HGT, are similar across open symbioses within guts of mammals[7], termites[49], and honey bees[50], in *A. fischeri* within the bobtail squid light organ[51], and *Curvibacter* species within the glycocalyx of *Hydra* species[52]. In closed symbioses, the shared features of genome reduction and lack of HGT are repeatedly observed for Bacteroidetes and Proteobacterial symbionts of various insect orders[18].

Caveats in categorizing symbioses.

Assigning symbioses as open, closed, or mixed is often clear-cut, but not always. Closed systems can be readily categorized when they are ancient, and exhibit pronounced genome reduction and divergent sequences[27]. But some younger symbioses that are strictly clonal have not reached these extreme states, as distinguishing genomic features emerge slowly. In early stages of a closed symbiosis, genomes typically accumulate recently inactivated pseudogenes, but the initial genome shrinkage is not abrupt[6,53]. This point is illustrated by a study comparing genomic features of symbiotic *Burkholderia gladioli* strains in the beetle *Lagria villosa*. Some have normal-sized genomes with few pseudogenes, are cultivable and able to infect plants, from which they are newly acquired by each beetle generation. But one strain (*B. gladioli* Lv-StB) has a somewhat reduced genome, abundant pseudogenes (1,149 pseudogenes and only 744 intact genes), and accelerated sequence evolution, pointing to a lifestyle shift to host-restriction and clonality[54]. Similarly, genomic analyses of marine bivalve symbionts reveal widely varying levels of genome reduction corresponding to the extent to which transmission is vertical versus horizontal[36], and open, mixed and closed lineages occur in the genus *Sodalis*, with radical consequences for genome size and architecture[6]. Despite uncertainties in categorizing every system, recognizing these categories allows insight into why symbiotic systems display different genomic features and routes to innovation.

Innovations in open symbioses

Open symbiotic systems enjoy many avenues for innovation, and also for deterioration. Strains can be lost and gained, and persisting residents can evolve through mutation, drift, selection, and recombination (Figure 1, Figure 4). Lineage evolution often features HGT, whereby a symbiont gains genetic material and associated functions from unrelated bacteria, potentially with consequences for hosts. Co-resident, conspecific strains can undergo homologous recombination, preventing or slowing mutation accumulation and avoiding the clonal interference that otherwise slows adaptive evolution. The relative contributions of these processes to innovation at the community level varies, and each process has the potential to either benefit or harm hosts.

Strain recruitment and loss.

In open symbioses, effects on host biology depend on the composition of the symbiotic community. Sometimes, many species or strains contribute to emergent phenotypes, such as polysaccharide digestion or protection from pathogens. Disturbance of complex communities of symbionts can have long-term consequences for host health. For example, mice that are experimentally fed a diet deficient in complex polysaccharides experience shifts in gut microbiome composition and functionality, including irreversible losses of certain polysaccharide-digesting strains[55]. These changes can leave hosts unable to digest complex polysaccharides, even if they are later re-introduced to the diet[55]. More generally, widespread antibiotic exposure is hypothesized to lead to disrupted gut microbiomes in populations of humans and honey bees, potentially impairing host health[56,57].

Hosts that depend on services of open microbial communities possess innovations to ensure that symbiotic strains are recruited and maintained. For both complex and simple communities, this can include behavioral adaptations. Transmission of gut microbial communities to other hosts through familial contact is common in hominids[58], termites[59], and social bees[60], and can produce signatures of co-evolution over long time periods[61–63]. In relationships where colonization occurs every generation, hosts possess innovations that allow them to filter potential colonizers so as to bar non-symbionts. The stinkbug *Riptortus pedestris* acquires bacterial symbionts from the soil at every generation. Although various bacteria enter the foregut, a specific constricted midgut region filters for motile *Burkholderia* symbionts and close relatives, then strain competition within the symbiotic organ results in an exclusive partnership[64,65]. Similarly, bobtail squids restrict colonization of the symbiotic organ by *A. fischeri* strains, in part by selecting strains on the basis of their beneficial activity of light production[66–69]. Strains use several strategies to compete within symbiotic crypts, sometimes forming stable strain mixtures in hosts[5,51,69]. In some cases, host adaptations may control symbiont proliferation, as appears to be the case for the *Hydra-Curvibacter* symbiosis. The 4.3 Mb genome of *Curvibacter* includes two quorum-sensing operons, and *Curvibacter* symbionts produce signalling molecules that are subsequently modified by host-encoded enzymes, resulting in dramatic shifts in symbiont gene expression and phenotype[52]. These shifts enable robust colonization of host tissues and modulation of the *Hydra* innate immune system, through reduced production of flagellin, a trigger for host Toll-like receptors.

Reliable colonization of hosts also depends on microbial adaptations. A survey of symbiont genomes, from systems in which symbionts are recruited from the environment, showed consistent presence of genes enabling both flagellum-based motility and chemotaxis; both functions are typically lost from most maternally-transmitted symbionts[70]. In zebrafish, gut bacteria colonize from surrounding water, and, in experimental populations selected for host colonization ability, enhanced motility was the dominant adaptation[71,72]. Another challenge for symbionts is the need to modulate immune responses triggered by bacterial cell envelope components; for example, *Bacteroides* in the human gut microbiota dampens inflammation by modifying cell surface molecules[73].

In open symbiotic communities, recruitment is governed not only by whether symbionts reach the symbiotic organ, but also by interactions within the microbial community, both

antagonistic and cooperative. Symbionts in open communities harbor extensive machinery devoted to competition for nutrients[74,75] and to weaponry for inter-strain and inter-species warfare[76]. For example, Type VI Secretion Systems, used to kill competing Gram negative bacteria, are abundant and diverse in *Bacteroidales* in the human gut[76] and in *Proteobacteria* in the bee gut[77] and are used by competing *A. fischeri* strains within host crypts[78]. Other mechanisms also mediate bacterial antagonism, with varying levels of target specificity. *E. coli* and other *Enterobacteriaceae* within the gut compete using microcins, peptides with potent toxicity for a restricted range of competing bacterial strains[79].

Evolution of resident strains.

Strain turnover is not the only mechanism for change in open systems. Persisting strains can evolve, sometimes over short timescales, within hosts. Analyses of genomic data for 40 dominant species in the human gut revealed that, in just a few months, strains underwent sequence evolution of existing genes, gene acquisition via HGT, and gene loss, and that certain novel variants spread rapidly, implying strong positive selection[20]. Strain evolution can be fast enough to be captured by laboratory experimental evolution approaches: commensal *E. coli* strains introduced into mouse guts experience bursts of adaptive evolution over very short time scales (months)[80,81]. Strains were found to rapidly evolve enormous variation in mutation rates due to mutations in repair genes, which accelerated strain divergence[81]. Beyond mutations in existing genes, experimental evolution studies in which multiple strains were present in mouse guts showed that strong selective sweeps can be seeded by phage-mediated HGT conferring adaptive traits, such as the ability to metabolize a new carbon source or resist antibiotics[80]. Other mutations, including deleterious ones, can hitchhike on positively selected haplotypes and then persist[81]. In the long-term, negative selection (against new deleterious mutations) is effective in preserving functions of ancestral genes, as shown by relative frequencies of changes at nonsynonymous and synonymous sites within protein-coding genes (dN/dS ratios)[20]. Furthermore, over long time scales, strain turnover in gut communities may limit the extent of within-host adaptation of strains[20].

Whole genome sequencing of multiple bacterial isolates from particular symbiont species has revealed that HGT, often involving bacteriophage or other mobile units, is the most potent source of novelty, generating distinct gene sets for individual strains[11,82]. Such strain-specific ‘accessory’ genes often confer new capabilities. One large-scale analysis of multiple strain genomes from species of *Bacteroides* (dominant and well-studied members of human gut microbiomes) revealed that strains share only several hundred core (universal) genes, but that pooled gene sets of all strains for a species (pan-genomes) can contain over 70,000 accessory genes[83]. *Bacteroides dorei* strains that are nearly identical for sequences of shared genes, contain hundreds of strain-specific genes, often associated with bacteriophage[84]. Similarly, 48 *Gilliamella apicola* genomes from honey bee guts encoded 1,480 core genes but 4,408 accessory genes[85,86]. Even single-symbiont systems, exemplified by *A. fischeri*, include many strains differing in accessory gene sets[5,51] (Figure 2A).

Repercussions of gene gain and loss.

Genomic sequencing, which enables specification of complete gene repertoires, has shown that HGT is implicated in every kind of symbiont adaptation in open symbioses, including changes related to colonizing hosts. For example, metagenomic analyses of the open symbiont communities of sponges revealed numerous phage-associated genes encoding ankyrin proteins, which are known to modulate cellular immune responses of diverse animal phyla[87]. Using both synthesized proteins and heterologous expression **[G]** in *E. coli*, researchers showed that these phage-encoded ankyrins increase bacterial persistence when exposed to mouse macrophages, and dampen transcriptomic signatures of immune responses that are widely conserved across animals. Based on analyses of genomes of bacterial gut symbionts, genes underlying toxin and secretion systems, which function in strain competition, are exchanged frequently among community members and are among the most dynamic genomic elements[77,88,89]. Such antagonistic interactions can result in exclusion of invaders, potentially protecting hosts from pathogenic infection.

HGT also introduces a regular influx of new enzymatic capability into open communities, permitting symbionts to better adapt to host ecology while potentially benefiting hosts. Gut bacteria of herbivorous or omnivorous hosts often secrete carbohydrate active enzymes (CAZymes) that degrade complex polysaccharides, providing access to the energy stored in plant cell wall components. In *Bacteroidales* spp. of human guts, strains have distinct repertoires of CAZyme loci[90,91], and similar variation occurs among *Gilliamella* and *Bifidobacterium* strains of honey bee guts[86,92]. HGT between *Spirochetes* species in termite gut communities enables digestion of complex plant polysaccharides[49]. A combination of genomic sequencing, strain isolation, and protein biochemistry was used to show that the specific polysaccharide utilization locus for digestion of the algal polysaccharide porphyran was transferred from marine bacteria to human gut *Bacteroides* in populations whose diets regularly include seaweed[93].

Although metagenomic sequencing can give insights into functional capabilities of bacterial communities as a whole, the typical short-read sequences do not resolve the frequency and range of gene movement among strains through HGT. However, new sequencing methods using long-reads or proximity ligation methods are beginning to give a clearer picture of HGT in open symbioses, as exemplified by a study showing rapid transfer of antibiotic resistance genes across species within gut communities of humans, both in presence and absence of antibiotic treatment[94].

Differences in gene sets among symbionts reflect strain-specific gene loss in addition to HGT. In densely packed, host-associated communities, strains may lose genes that become superfluous due to the metabolic contributions of other community members. A result is that strains or species rely on one another for essential metabolites, a relationship referred to as syntrophy or cross-feeding[95,96]. Co-dependence that arises from complementary gene losses, termed ‘Black Queen’ evolution[97], may help to stabilize community composition. Based on reconstruction of genomes from metagenomic data for as-yet uncultivated strains in the human gut microbiome, individual strains often lack widely conserved biosynthetic pathways for vitamins, amino acids and essential fatty acid components of membranes,

suggesting their uptake from other community members[7]. Such co-dependent symbiotic communities are vulnerable to invasion by strains that reap benefits but do not contribute, making cooperative communities less stable than competitive ones[95,96]. Potentially, host adaptations might stabilize such communities, by supporting persistent spatial clustering of cooperating cell lineages: this possibility is supported by recent experimental evolution studies of reciprocally dependent *E. coli* strains which demonstrated that such clustering promoted cooperation[98]. Spatial clustering could be enhanced by host anatomical features or behaviors (such as trophallaxis **[G]**) that promote co-transmission.

Innovations in closed symbioses

Transmission modes that enforce clonality, such as strict maternal inheritance, result in long term degradation of symbiont genomes with ongoing gene loss and little or no HGT (Figure 5). The extent of symbiont genome reduction can be drastic, even for extracellular symbionts, as illustrated by a recent analysis of the tiny genomes (215–310 kb) of the maternally transmitted, extracellular *Stammera* symbionts of tortoise beetles that provide pectin-digesting enzymes used for digesting dietary plant fiber[31,99]. Ongoing gene losses mean that, from the symbiont perspective, the association is a one-way street: established symbionts cannot revert to free-living lifestyles, and cannot even switch to different host lineages. These ancient closed symbioses present a conundrum: how are these deteriorating symbioses maintained, and how do they respond to changing ecological conditions?

Limitations to genomic decay.

Genome decay, with different levels of severity, repeats itself in many closed symbioses; the constellation of changes, called the ‘symbiosis rabbit hole’, is perhaps the most distinctive genomic syndrome in prokaryotes[12]. These characteristic genomic features, reviewed previously, include tiny genomes with few genes and accelerated sequence evolution[3,6,18,27]. The functional losses include decay of central cellular functions, as exemplified by thermally unstable gene products, loss of DNA repair capabilities, minimal sets of tRNA synthetases, impaired tRNA processing[100,101], and lowered translational efficiency[102]. Genome degradation often seems to be accelerated by the loss of DNA repair genes and consequent increase in mutation rates, which are typically elevated to varying degrees in heritable symbionts[40,43].

One limit to genome reduction in obligate, heritable symbionts is the number of genes required to serve host needs. For example, symbionts of *Paracatenula* flatworms contribute relatively complex functions involving energy production and storage, and retain relatively large genomes (1.34 Mb), despite the estimated age of 500 My for this symbiosis[8]. By contrast, *Sulcia muelleri* symbionts in sap-feeding insects are also ancient (~280 My), but have genomes of 0.15–0.28 Mb; the larger genome supplies eight essential amino acids and the smaller only three[27,103]. Sometimes a single symbiont retains genes underlying a variety of functions beneficial to hosts; thus, *Profftella* symbionts of the psyllid *Diaphorina citri* synthesize vitamins and carotenoids, as well as polyketide toxins that function in host defense[104,105]. In another example, the reduced genome (~500 kb) symbionts of reed beetles (Donaciinae) alternate between provisioning amino acids during larval stages and

secreting digestive pectinases during adult stages, and transcriptome analyses show that the underlying genes are expressed at corresponding beetle life stages[106].

Innovations to ensure transmission.

When host progeny require symbionts for survival, hosts show adaptations to ensure transmission, as revealed by a variety of microscopy methods, often using fluorescent *in situ* hybridization to resolve symbiont cells[17]. In gut-inhabiting obligate symbionts, mothers sometimes deposit an inoculum on or near eggs, to be ingested by newly hatched progeny. This transmission route can involve striking adaptations: in the extracellular midgut symbionts of stinkbugs (family Urostylididae), large ovaries produce a voluminous jelly-like substance that contains symbiont inocula as well as nutrition upon ingestion by hatchlings[33]. Plataspid stinkbug females produce massive amounts of a specialized protein that is deposited with the reduced genome symbiont, *Ishikawaella*, during transmission within maternally produced capsules; RNAi knockdown of this host protein results in transmission failure[34]. Many other heritable symbionts colonize eggs or progeny within the mother's body, using a variety of routes. In whiteflies, remarkably, an entire maternal bacteriocyte, containing a nuclear genome as well as resident *Portiera* symbionts, is transferred into the egg; sequencing of germ line and bacteriocyte genomes show that the transferred bacteriocyte persists throughout development and forms a genetic lineage divergent from the main germ-line lineage[107]. More often, symbionts are transmitted to eggs or embryos as microbial inocula. This transfer can be largely host-controlled, as in aphids in which the passive *Buchnera* symbionts lack flagella and mobility[30]. Alternatively, transmission can require symbiont participation, as in tsetse flies in which transcriptomic and immunohistochemistry analyses show that *Wigglesworthia* symbionts activate flagellar motility machinery to colonize developing larvae via maternal milk glands[108]. In some hosts, symbionts must colonize different tissues during host development. For example, *Sodalis pierantonus* moves between larval and adult bacteriomes during development of cereal weevil hosts. A combination of bacteriocyte imaging and RNA sequencing revealed that this movement is achieved through a coordinated sequence of changes in host and symbiont gene expression and cellular features, including migration of the larval bacteriocytes to a new location, and activation of the symbiont Type III secretion system machinery upon colonization of the adult bacteriocytes[109].

Innovations to compensate for genomic degradation.

The one-way ratchet towards ever more genomic degradation and loss of function can lead to extreme outcomes, as has been elucidated recently by large scale genome sequencing of symbionts in many closed symbioses. In *Buchnera* symbionts of aphids, sequencing of genomes from across the host phylogeny reveals an unrelenting ratchet of gene loss in each lineage, with this loss more pronounced for some loci and some lineages[42]. Likewise, in cicadas, genomes of the symbiont *Hodgkinia* often incur deletions of essential genes, requiring hosts to maintain multiple *Hodgkinia* genomes with complementary gene sets[110,111].

How do closed symbioses persist, despite ongoing losses of genes and functions? Some endosymbiont genomes encode nearly complete biosynthetic pathways, with only a single

enzyme not encoded, suggesting that another gene has expanded function to complete the missing step[4]. While functional studies of non-cultivable symbionts are challenging, one approach to study gene function is to use heterologous expression in a laboratory model. Using heterologous expression in *Escherichia coli* missing the same gene, a *Buchnera* enzyme from the branched chain amino acid pathway was shown to have expanded its substrate affinity so as to complete a missing step in pantothenate biosynthesis[112]. Thus, promiscuous enzyme activities may sometimes enable a reduced genome to retain capabilities.

Even for genes that are retained, ongoing mutation accumulation in closed systems results in thermal instability of proteins, such that symbionts are highly heat sensitive, which can in turn limit the thermal range of the host[113,114]. A conspicuous feature, observed repeatedly for symbionts in closed systems, is the constitutive overexpression of molecular chaperones, including GroEL[115], which has been shown to compensate for effects of destabilizing mutations[116]. Proteomic analyses of *Buchnera* cells using mass spectrometry show that GroEL constitutes up to 10% of protein, and other chaperones are also abundant[117].

Functional novelty in closed symbioses.

In closed symbioses, adaptations to preserve the symbiosis largely fall to the host, and recent discoveries show two surprising routes (Figure 5). First, genome and transcriptome sequencing has revealed that hosts themselves acquire horizontally transferred bacterial genes that are expressed exclusively or primarily in bacteriocytes (Supplementary Table 1). For example, mealybugs harboring *Tremblaya* and *Moranella* and aphids harboring *Buchnera* have acquired genes underlying biosynthesis or recycling of peptidoglycan components[118–120]. Many leafhoppers possess two bacterial symbionts, each housed in a distinct bacteriocyte type characterized by distinct gene expression profiles, which are predicted to complement the capabilities of the resident symbiont type. These bacteriocyte-specific genes include numerous genes acquired through horizontal transfer from bacteria, as well as ancestral host genes that seem to acquire novel functions in bacteriocytes[121].

A second evolutionary route to innovation by hosts in closed symbioses is the gain of new microbial partners that retain intact pathways for supporting themselves and their hosts (Supplementary Table 2). A combination of genome sequencing, transcriptome analyses, and phylogenetic reconstruction shows that these new symbionts may supplement or supplant ancient symbionts. For example, in cixiid planthoppers, two ancient symbionts (*Sulcia* and *Vidania*) with tiny genomes (157 kb and 136 kb, respectively) are joined by a novel symbiont, *Purcelliella* (Enterobacterales)[103]. *Purcelliella* is closely related to plant pathogens and retains a somewhat larger genome (480 kb) that encodes pathways for biosynthesis of B vitamins and of cysteine, the latter of which may complement metabolites needed for methionine synthesis by *Vidania*. Likewise, multiple cicada lineages have replaced their *Hodgkinia* symbionts, which have fragmented and deteriorated genomes, with symbiotic fungi[122]. In lachnine aphids, *Buchnera* is co-resident with *Serratia symbiotica* strains that have taken over amino acid biosynthesis functions, and the acquisition of this novel symbiont has enabled further erosion of the *Buchnera* genomes[123]. Blood-feeding ticks rely on bacterial endosymbionts for B vitamin biosynthesis, and some tick species

have replaced the more ancient *Coxiella* symbiont with a *Franciscella* partner experimentally demonstrated to serve this function[124]. Potentially, replacing an ancient, degraded symbiont with a more robust one can trigger loss of host support mechanisms. Thus, sharpshooters (Cicadellinae) have replaced the ancient *Nasuia* symbiont with a newer arrival (*Baumannia*), and transcriptome studies of the distinct bacteriocyte types show that those housing *Baumannia* express fewer host genes predicted to assist symbionts with cell envelope generation and central information processing[125].

In some cases, host genes have undergone adaptation to control and support symbionts with highly reduced capabilities. For example, *Buchnera* receives non-essential amino acid substrates abundant in the aphid diet and returns essential amino acids required by hosts. But *Buchnera* genomes have lost genes for membrane-bound transporters; instead, immunolocalization studies show that host-encoded transport proteins are localized to both the bacteriocyte membrane and the host-derived 'symbiosomal' membrane enclosing each *Buchnera* cell[126]. Furthermore, expression of these transport proteins in frog oocytes revealed their capacity to transport multiple amino acids between the insect body cavity, bacteriocyte cytoplasm, and the symbiosomal space surrounding each symbiont cell, in some cases using feed-back regulation to adjust the movement based on host needs[4,126,127].

Innovations to evade immune responses.

A challenge for all animal-bacterial symbioses is that of establishing stable, regulated populations despite innate immune pathways, which are universal in animals and are triggered by widespread components of bacterial cell envelopes. Obligate heritable symbioses often have solved this challenge through unusual modifications in hosts. One apparent solution, found in aphids, is the elimination or reduction of innate immune capabilities, as revealed by absence of many immune-related genes from the sequenced pea aphid genome[128], as well as a lack of the usual insect immune responses following experimental challenge[129]. Additionally, aphid enzymes AmiD and LdcA, acquired by HGT from bacteria and expressed in bacteriocytes, are predicted to degrade peptidoglycan components, and acquisition and expression of these genes has been hypothesized as a host adaptation to suppress remaining immune responses. However, RNA interference to knock down expression of these genes reduced *Buchnera* numbers, which suggests that these HGT products support *Buchnera* growth[130].

A constitutive reduction of innate immunity is likely only possible for organisms, such as aphids, that use largely sterile diets (phloem sap) and have short lifespans that minimize pathogen impacts. In contrast to aphids, cereal weevils maintain a complete set of innate immune pathways, but express a bacteriocyte-specific isoform of peptidoglycan recognition protein (PGRP); the bacteriocyte PGRP isoform was shown experimentally to cleave tracheal cytotoxin (TCT), a symbiont-derived peptidoglycan component that otherwise causes a systemic immune response[131]. Furthermore, experiments using RNA interference to knock down PGRP resulted in TCT escape from the bacteriome and a deleterious systemic immune response.

On the symbiont side, evasion of immune responses may be accomplished in part by adaptive gene loss: one of the most depleted gene categories in obligate symbionts is that

underlying synthesis of cell envelope components, including peptidoglycan components and outer membrane proteins that typically trigger immune responses[27]. In several cases in which a more ancient and more recent symbiont reside within a host, microscopy studies combined with genome sequencing have revealed that the more recent symbionts, which retain normal Gram negative cell walls, sequester themselves within the cytoplasm of the more ancient symbiont that lack cell wall components[132–134]. These rare instances of a bacterium living within another bacterium may represent mechanisms by which a new symbiont can avoid host immune receptors.

Innovations in mixed symbioses

Similar to symbionts in closed systems, symbionts in mixed systems are predominantly vertically transmitted and clonal within their hosts. However, they are also occasionally transferred to other host lineages, of the same or different species. Upon arrival in a new host, symbiont success depends on genetic innovations that allow them to evade the host immune response, to replicate without excessive virulence, to achieve vertical transmission, and to alter hosts in order to increase the frequency of infected matrilines (Figure 6).
The best-studied and most widespread such group is *Wolbachia*. Phylogenomic analyses show that the success of *Wolbachia* has depended on its capacity for horizontal transfer between arthropod species and frequent HGT enabling acquisition of symbiont-beneficial genes[135,136]. Other examples include lineages within *Hamiltonella, Riesia*, *Arsenophonus*, *Sodalis*, *Spiroplasma*, *Serratia*, and *Rickettsia*. Based on surveys to date, mixed symbioses are concentrated in terrestrial arthropods, including diverse insects and ticks and including many species important as disease vectors, agricultural pests, or beneficial biocontrol agents.

Innovations for establishment and spread.

Mechanisms for achieving vertical transmission vary among symbiont groups. Experiments with mutant *Drosophila melanogaster* lacking a functional yolk protein receptor revealed that *Spiroplasma* symbionts invade eggs via a conserved pathway for endocytosis of yolk protein, a route that may be used by other symbionts as well[137]. Sometimes, symbionts co-opt the transmission routes of more ancient obligate symbioses, as in facultative symbionts of aphids that enter progeny via the route used by the obligate symbiont, *Buchnera*[30,138]. Other bacterial lineages have repeatedly managed to enter new hosts: a prime example is *Sodalis*, a clade that has formed independent, maternally-transmitted symbioses in diverse insects, including tsetse flies[108], grain weevils[131], spittlebugs[139], and mealybugs[133]. This repeated success at symbiotic life reflects a preadaptation: when an isolate of the proto-symbiont *Sodalis praecaptivus* is experimentally introduced to tsetse flies, it uses quorum sensing to attenuate virulence, enabling host survival and transmission to progeny[140].

Highly successful symbionts in mixed systems possess a variety of genetic innovations that enable them to increase the proportion of infected matrilines within host populations (Figure 6). Many, including *Wolbachia, Rickettsia*, and *Spiroplasma* in arthropods, act as reproductive manipulators. They shift progeny sex ratios towards females, kill sons, or cause infected males to sterilize uninfected females[141,142]. The underlying mechanisms are diverse. For example, within *Drosophila* hosts, both *Spiroplasma* and *Wolbachia* target the X

chromosome dosage compensation mechanisms to selectively kill male progeny but employ different mechanisms; *Spiroplasma* use an ankyrin-associated peptide toxin[142,143], but the mechanisms are still unclear for *Wolbachia*, even though the responsible genes have been experimentally identified and shown to have varying potencies[144,145]. Recent comparative genomic analyses, discussed in published[146] and preprint[147] articles, show that these genes evolve rapidly and undergo frequent phage-mediated HGT.

Another symbiont strategy for expanding the proportion of infected matrilines is to provide direct fitness advantages to female hosts; this common effect is often combined with reproductive manipulation. These fitness advantages fall into two main categories: defense against parasites and nutritional support. In contrast to most closed systems, symbionts in mixed systems often defend hosts against natural enemies, swapping out novel mechanisms to meet the dynamic 'arms-race' nature of host-parasite coevolution. One method for demonstrating these effects is pathogen challenges that compare susceptibility of uninfected hosts with that of genetically similar hosts experimentally infected with a symbiont. This approach has shown that heritable symbiont-based defense against parasites or pathogens is widespread in insect symbioses. Examples include protection by *Wolbachia* against insect viruses[46,47], by *Spiroplasma* against parasitic nematodes[148], and by *Hamiltonella defensa* against aphid parasitoids[149–151]. These protective mechanisms are diverse but are usually based on genes acquired through HGT. Thus, the *Spiroplasma* symbionts of some *Drosophila* spp. have acquired varying repertoires of ribosome-inactivating proteins that protect hosts against both parasitic nematodes and wasps[148].

Mobile gene pools in mixed symbioses.

A ubiquitous feature of symbionts in mixed systems is their ability to pick up new capabilities and quickly adapt — whether to benefit hosts by adopting new defenses against natural enemies, or to harm hosts by overcoming host resistance to reproductive manipulation. Comparative genome analyses point to a mobile gene pool shared among distant symbiont lineages, which have the opportunity to exchange genes within coinfected hosts. For example, the complete genomes of *H. defensa* and *Arsenophonus nasoniae* share numerous HGT cassettes that are also present in other insect symbionts[41,152].

In the case of *H. defensa*, defense of aphid hosts against parasitoid wasps depends on phage that jump among symbiont strains. Recent comparative genomic studies reveal that the phage themselves undergo extensive exchange of gene cassettes that encode toxins active against eukaryotic parasites, including homologs of cytolethal distending toxin (CdtB)[44,153]. Remarkably, the gene encoding CdtB is sometimes transferred to the host nuclear genome, as observed in some aphids and some *Drosophila* spp., suggesting that the defensive machinery is deployed directly by the host[154]. Likewise, genome sequencing surveys of the bacteriophage WO, which is central to *Wolbachia*'s adaptations for reproductive parasitism, show that WO is responsible for transferring the genes underlying both reproductive incompatibility[146] and male-killing[155].

Symbionts in mixed systems sometimes supply nutrients to hosts, while also exerting selfish effects. In *Hamiltonella* and *Arsenophonus* strains living in whitefly species, the nutritional provisioning itself reduces proportions of sons, as demonstrated by experiments

that manipulate symbiont titers and nutritional status. Thus, the same process confers dual symbiont fitness advantages, increasing overall host fecundity while also biasing towards daughters[156,157]. In general, the genes underlying nutrient provisioning are stable within symbiont genomes and represent widespread bacterial biosynthetic pathways, retained from non-symbiont ancestors. But even genes underlying nutritional functions can jump between symbiont species, as genomic analyses have revealed for vitamin-biosynthetic genes in *Erwinia*, *Sodalis*, and *Hamiltonella* symbionts in insects[158].

Conclusions and Future Perspectives

The success of symbiotic relationships, including their ability to overcome changing environmental conditions, depends on genetic innovations accrued by either partner. Symbiont innovations include those that allow them to more successfully invade or compete in hosts or to influence host biology in ways that favor their own spread. Host innovations may allow for better transmission of beneficial symbionts to offspring, or for better maintenance, support, and control of symbionts.

Genomic sequencing has shown that symbiotic relationships evolve under the constraints of the underlying symbiont population structure, and that the symbiont transmission route has major consequences for the kinds of genetic innovations available. In open systems, hosts freely sample diverse bacterial strains and genes from the environment, and innovate by gain or loss of trait-bearing symbionts. Likewise, symbionts, as members of diverse pools, innovate by recombination including HGT, often mediated by phage. By contrast, symbionts in closed systems are strictly clonal and evolve largely through gene loss and genomic decay, leaving hosts with no other choice but to provide support to, or to replace, their symbiont. And in mixed systems, symbionts are mostly clonal, but occasional horizontal transmission allows hosts to gain new symbionts and allows host-associated symbionts to acquire genes through HGT, often from one another.

The study of animal-microbe symbioses has been complicated by intractability of most hosts and symbionts. Bacterial culture has long been a prerequisite for common genetic manipulation tools, such as mini-Tn7, recombineering, and CRISPR/Cas9. However, many symbionts, especially those that reside intracellularly, have complex nutritional or environmental requirements that make them resistant to cultivation[9]. Several common approaches have been adopted to overcome these limitations (Figure 7). In some cases, genomic data and empirical approaches have elucidated symbiont metabolism and thereby informed the development of axenic culture **[G]** media[9,159,160]. In other studies, insect cell lines have been successfully used to culture symbionts, facilitating sequencing of symbionts that reside at low densities in their host[44,161] and providing validation of genes underlying symbiotic functions. Analyses of *H. defensa* cultured in insect cell lines have confirmed phage toxins as the active killers of parasites of insect hosts, for example[150]. Other productive approaches include heterologous expression of symbiont gene products and experimental evolution studies, in which genetic changes in symbiont populations can be directly observed[87,112].

Genetic manipulation of bacteria is commonly accomplished by conjugation of plasmids from a donor to recipient. Conjugal or transduction-based strategies have succeeded for some culturable symbionts, including *S. glossinidius*[162,163], and have enabled validation of genes involved in establishing symbiotic interactions[140]. Conjugation has also been used to produce *Asaia* and *Arsenophonus* strains with integrated fluorescent proteins for *in-vivo* tracking of infections in insect hosts[164,165]. While culturability has long been a prerequisite for reliable conjugation, recent approaches with a single delivery vector now allow for *in situ* microbial genetics targeting specific DNA sequences or community members, as shown in one published study and one preprint[166,167]. We are also witnessing an extension of genetic tools to non-model, host-associated bacteria, producing resources that promise to facilitate studies of symbiont innovations in alternative host communities[10,168–170].

Advances in genomic sequencing have complemented experimental approaches to provide a better basic understanding of the genetic innovations underlying symbioses. Symbiont effects on host phenotypes have been identified by transfection of uninfected hosts, as for aphid symbionts, in which microinjection into hosts results in stably infected matrilines with altered resistance to parasites or to heat stress[171,172]. Transfection has also enabled the development of symbiont-based biotechnology for practical purposes, such as fighting animal and plant diseases. *Wolbachia* strains that act as reproductive manipulators and suppress viral load have been exploited to reduce the capacity of crop pest insects to vector plant viruses[173], and the capacity of mosquitos to vector human dengue virus[174] and also to suppress vector population numbers via male sterility[175]. In other cases, the ability of symbiotic bacteria to colonize and persist within hosts makes them attractive chassis organisms for the delivery of synthetic pathways, thereby acting as living therapeutics. Recently, commensal *E. coli* of the human gut have been engineered to detect inflammation, and for mitigating IBD and phenylketonuria[176–178]. In mosquitos, bacterial symbionts have been genetically manipulated to express anti-*Plasmodium* compounds that reduce vectoring capacity[179]. And in honey bees, a specialized gut bacterium engineered to express dsRNA was able to prime the RNAi pathway of bees to protect them against viruses and mites that are major causes of bee decline[180].

Although large-scale metagenomic sequencing is a major source of our knowledge of animal symbioses, the usual short-read metagenomic data cannot readily resolve genetic changes in individual symbiont lineages. Some new developments, including long-read sequencing, experimental evolution approaches, and genetic engineering of non-culturable organisms, are just beginning to be applied to the study of symbioses and will enable finer scale elucidation of these changes. Likewise, studies of host innovations for symbiosis are relatively few, as non-model animals are often a challenge for genetic studies. This gap is starting to be filled. For example, a genome-enabled study of gene expression in the two symbiotic organs in the bobtail squid revealed distinct genetic underpinnings[181], and other studies have begun to elucidate the genetics and development of host organs that house symbionts[182]. Far better genome assemblies for hosts are now feasible, enabled by proximity ligation and long-read sequencing (for example,[183]). These approaches, combined with experimental work, will help to illuminate the host's role in maintaining symbiotic partnerships. Thus, we can look forward to an ever-clearer picture of the innovations and constraints that govern the evolution of symbioses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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GLOSSARY

Symbiont transmission mode
The route by which symbionts are acquired each generation, ranging from strictly vertical (parent-to-offspring) to strictly horizontal (between non-parent-offspring pairs of hosts or between hosts and non-host sources). Mixed-mode transmission combines vertical and horizontal modes.

Genetic recombination
The exchange of genetic material between organisms. Recombination can be roughly classified as: homologous recombination, which involves exchange of related sequences; and non-homologous recombination, in which unrelated sequences are inserted into the genome, as in the case of horizontal gene transfer (HGT).

Purifying selection
The removal of deleterious alleles by natural selection. Also referred to as negative selection. This is the most common form of selection, as mutations are more often deleterious than beneficial.

d*N*/d*S*
The ratio of nonsynonymous substitutions (that is, those that change the amino acid sequence) per non-synonymous site (d*N*) to the number of synonymous substitutions (that is, those that do not change the amino acid sequence) per synonymous site (d*S*), used to determine the mode and strength of selection that has acted on genetic sequences.

Bacteriocytes
Host cells that are specialized for housing bacterial symbionts.

Heterologous expression
Expression of a gene in an alternative, genetically tractable host.

Trophallaxis
The exchange of food through an oral-to-oral or fecal-to-oral transmission route, commonly performed by members of the same community.

Axenic culture
The culture of a single microbial strain, in the absence of additional strains or hosts, in laboratory culture media.

REFERENCES

1. McFall-Ngai M et al. Animals in a bacterial world, a new imperative for the life sciences. Proc. Natl. Acad. Sci. U. S. A 110, 3229–3236 (2013). [PubMed: 23391737]
2. Moran NA, McCutcheon JP & Nakabachi A Genomics and evolution of heritable bacterial symbionts. Annu. Rev. Genet 42, 165–190 (2008). [PubMed: 18983256]
3. Wernegreen JJ Ancient bacterial endosymbionts of insects: Genomes as sources of insight and springboards for inquiry. Exp. Cell Res 358, 427–432 (2017). [PubMed: 28454877]
4. Shigenobu S & Wilson ACC Genomic revelations of a mutualism: the pea aphid and its obligate bacterial symbiont. Cell. Mol. Life Sci 68, 1297–1309 (2011). [PubMed: 21390549]
5. Bongrand C et al. Using colonization assays and comparative genomics to discover symbiosis behaviors and factors in *Vibrio fischeri*. mBio 11, e03407–19 (2020). [PubMed: 32127462]
6. McCutcheon JP, Boyd BM & Dale C The life of an insect endosymbiont from the cradle to the grave. Curr. Biol 29, R485–R495 (2019). [PubMed: 31163163]
7. Nayfach S, Shi ZJ, Seshadri R, Pollard KS & Kyrpides NC New insights from uncultivated genomes of the global human gut microbiome. Nature 568, 505–510 (2019). [PubMed: 30867587]
8. Jäckle O et al. Chemosynthetic symbiont with a drastically reduced genome serves as primary energy storage in the marine flatworm *Paracatenula*. Proc. Natl. Acad. Sci. U. S. A 116, 8505–8514 (2019). [PubMed: 30962361] The intracellular symbiont of *Paracatenula* flatworms possesses a highly reduced genome (1.34 Mb) and represents the oldest documented animal symbiosis (500 My); this article shows that it provisions its host through outer membrane vesicle secretion.
9. Masson F & Lemaitre B Growing ungrowable bacteria: Overview and perspectives on insect symbiont culturability. Microbiol. Mol. Biol. Rev 84, e00089–20 (2020). [PubMed: 33177190]
10. Elston KM, Leonard SP, Geng P, Bialik SB & Barrick JE Engineering insects from the endosymbiont out. Trends Microbiol 29, Jun 5:S0966–842X(21)00126–8 (2021).
11. Kirchberger PC, Schmidt M & Ochman H The ingenuity of bacterial genomes. Annu. Rev. Microbiol 74, 815–834 (2020). [PubMed: 32692614]
12. Bennett GM & Moran NA Heritable symbiosis: The advantages and perils of an evolutionary rabbit hole. Proc. Natl. Acad. Sci. U. S. A 112, 10169–10176 (2015). [PubMed: 25713367]
13. Husnik F & Keeling PJ The fate of obligate endosymbionts: reduction, integration, or extinction. Curr. Opin. Genet. Dev 58–59, 1–8 (2019).
14. Sudakaran S, Kost C & Kaltenpoth M Symbiont acquisition and replacement as a source of ecological innovation. Trends Microbiol 25, 375–390 (2017). [PubMed: 28336178]
15. Husnik F & McCutcheon JP Functional horizontal gene transfer from bacteria to eukaryotes. Nat. Rev. Microbiol 16, 67–79 (2018). [PubMed: 29176581]
16. Salem H, Florez L, Gerardo N & Kaltenpoth M An out-of-body experience: the extracellular dimension for the transmission of mutualistic bacteria in insects. Proc. Biol. Sci 282, 20142957 (2015). [PubMed: 25740892]
17. Bright M & Bulgheresi S A complex journey: transmission of microbial symbionts. Nat. Rev. Microbiol 8, 218–230 (2010). [PubMed: 20157340]
18. McCutcheon JP & Moran NA Extreme genome reduction in symbiotic bacteria. Nat. Rev. Microbiol 10, 13–26 (2011). [PubMed: 22064560]
19. Foster KR, Schluter J, Coyte KZ & Rakoff-Nahoum S The evolution of the host microbiome as an ecosystem on a leash. Nature 548, 43–51 (2017). [PubMed: 28770836]
20. Garud NR, Good BH, Hallatschek O & Pollard KS Evolutionary dynamics of bacteria in the gut microbiome within and across hosts. PLoS Biol 17, e3000102 (2019). [PubMed: 30673701] This study used a novel metagenomics-based approach to study the evolution of 40 bacterial species in the human gut and found signatures of within-host adaptation occurring over short timescales (6 to 12 months).
21. Bobay L-M & Raymann K Population genetics of host-associated microbiomes. Curr. Mol. Biol. Rep 5, 128–139 (2019).
22. Van Rossum T, Ferretti P, Maistrenko OM & Bork P Diversity within species: Interpreting strains in microbiomes. Nat. Rev. Microbiol 18, 491–506 (2020). [PubMed: 32499497]

23. Kikuchi Y, Ohbayashi T, Jang S & Mergaert P *Burkholderia insecticola* triggers midgut closure in the bean bug *Riptortus pedestris* to prevent secondary bacterial infections of midgut crypts. ISME J 14, 1627–1638 (2020). [PubMed: 32203122]
24. de Oliveira BFR, Freitas-Silva J, Sánchez-Robinet C & Laport MS Transmission of the sponge microbiome: moving towards a unified model. Environ. Microbiol. Rep 12, 619–638 (2020). [PubMed: 33048474]
25. Ansorge R et al. Functional diversity enables multiple symbiont strains to coexist in deep-sea mussels. Nat. Microbiol 4, 2487–2497 (2019). [PubMed: 31611646]
26. Picazo DR et al. Horizontally transmitted symbiont populations in deep-sea mussels are genetically isolated. ISME J 13, 2954–2968 (2019). [PubMed: 31395952]
27. Moran NA & Bennett GM The tiniest tiny genomes. Annu. Rev. Microbiol 68, 195–215 (2014). [PubMed: 24995872]
28. Gruber-Vodicka HR et al. *Paracatenula*, an ancient symbiosis between thiotrophic Alphaproteobacteria and catenulid flatworms. Proc. Natl. Acad. Sci. U. S. A 108, 12078–12083 (2011). [PubMed: 21709249]
29. Baker LJ et al. Diverse deep-sea anglerfishes share a genetically reduced luminous symbiont that is acquired from the environment. eLife 8, e47606 (2019). [PubMed: 31571583]
30. Koga R, Meng X-Y, Tsuchida T & Fukatsu T Cellular mechanism for selective vertical transmission of an obligate insect symbiont at the bacteriocyte–embryo interface. Proc. Natl. Acad. Sci. U. S. A 109, E1230–E1237 (2012). [PubMed: 22517738]
31. Salem H, Bauer E, Kirsch R, Berasategui A & Cripps M Drastic genome reduction in an herbivore's pectinolytic symbiont. Cell 171, 1520–1531 (2017). [PubMed: 29153832]
32. Mondal SI et al. Reduced genome of the gut symbiotic bacterium "*Candidatus* Benitsuchiphilus tojoi" provides insight into its possible roles in ecology and adaptation of the host insect'. Front. Microbiol 11, 840 (2020). [PubMed: 32435239]
33. Kaiwa N et al. Symbiont-supplemented maternal investment underpinning host's ecological adaptation. Curr. Biol 24, 2465–2470 (2014). [PubMed: 25264255]
34. Koga R et al. Host's guardian protein counters degenerative symbiont evolution. Proc. Natl. Acad. Sci. U. S. A 118, e2103957118 (2021). [PubMed: 34161284]
35. Kehr J-C & Dittmann E Protective tunicate endosymbiont with extreme genome reduction. Environ. Microbiol 17, 3430–3432 (2015). [PubMed: 26201437]
36. Russell SL et al. Horizontal transmission and recombination maintain forever young bacterial symbiont genomes. PLoS Genet 16, e1008935 (2020). [PubMed: 32841233] This study sequenced chemosynthetic symbionts from a variety of bivalve hosts in which transmission modes ranged from strictly horizontal to almost entirely vertical. Results revealed corresponding variation in the extent of genome erosion and rates of homologous recombination.
37. George EE et al. Highly reduced genomes of protist endosymbionts show evolutionary convergence. Curr. Biol 30, 925–933 (2020). [PubMed: 31978335]
38. Vavre F, Fleury F, Lepetit D, Fouillet P & Boulétreau M Phylogenetic evidence for horizontal transmission of *Wolbachia* in host-parasitoid associations. Mol. Biol. Evol 16, 1711–1723 (1999). [PubMed: 10605113]
39. Russell JA, Latorre A, Sabater-Muñoz B, Moya A & Moran NA Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea. Mol. Ecol 12, 1061–1075 (2003). [PubMed: 12753224]
40. Gerth M et al. Rapid molecular evolution of *Spiroplasma* symbionts of *Drosophila*. Microb. Genom 7, 000503 (2021).
41. Frost CL et al. The hypercomplex genome of an insect reproductive parasite highlights the importance of lateral gene transfer in symbiont biology. mBio 11, e02590–19 (2020).
42. Chong RA, Park H & Moran NA Genome evolution of the obligate endosymbiont *Buchnera aphidicola*. Mol. Biol. Evol 36, 1481–1489 (2019). [PubMed: 30989224]
43. Bourguignon T et al. Increased mutation rate is linked to genome reduction in prokaryotes. Curr. Biol 30, 3848–3855.e4 (2020). [PubMed: 32763167]

44. Chevignon G, Boyd BM, Brandt JW, Oliver KM & Strand MR Culture-facilitated comparative genomics of the facultative symbiont *Hamiltonella defensa*. Genome Biol. Evol 10, 786–802 (2018). [PubMed: 29452355]
45. Russell SL, Corbett-Detig RB & Cavanaugh CM Mixed transmission modes and dynamic genome evolution in an obligate animal–bacterial symbiosis. ISME J 11, 1359–1371 (2017). [PubMed: 28234348]
46. Asselin AK, Villegas-Ospina S, Hoffmann AA, Brownlie JC & Johnson KN Contrasting patterns of virus protection and functional incompatibility genes in two conspecific *Wolbachia* strains from *Drosophila pandora*. Appl. Environ. Microbiol 85, (2019).
47. Martinez J et al. Symbiont strain is the main determinant of variation in *Wolbachia*-mediated protection against viruses across *Drosophila* species. Mol. Ecol 26, 4072–4084 (2017). [PubMed: 28464440]
48. Newton ILG & Rice DW The Jekyll and Hyde symbiont: Could *Wolbachia* be a nutritional mutualist? J. Bacteriol 202, e00589–19 (2020). [PubMed: 31659008]
49. Tokuda G et al. Fiber-associated spirochetes are major agents of hemicellulose degradation in the hindgut of wood-feeding higher termites. Proc. Natl. Acad. Sci. U. S. A 115, E11996–E12004 (2018). [PubMed: 30504145]
50. Kwong WK & Moran NA Gut microbial communities of social bees. Nat. Rev. Microbiol 14, 374–384 (2016). [PubMed: 27140688]
51. Bongrand C & Ruby EG Achieving a multi-strain symbiosis: strain behavior and infection dynamics. ISME J 13, 698–706 (2019). [PubMed: 30353039] The squid light organ symbiosis involves only a single bacterial species, but this study, based on experimental colonization of hosts with combinations of isolates, reveals distinct bacterial strategies for competing and persisting within hosts.
52. Pietschke C et al. Host modification of a bacterial quorum-sensing signal induces a phenotypic switch in bacterial symbionts. Proc. Natl. Acad. Sci. U. S. A 114, E8488–E8497 (2017). [PubMed: 28923926]
53. Lo W-S, Huang Y-Y & Kuo C-H Winding paths to simplicity: genome evolution in facultative insect symbionts. FEMS Microbiol. Rev 40, 855–874 (2016). [PubMed: 28204477]
54. Waterworth SC et al. Horizontal gene transfer to a defensive symbiont with a reduced genome in a multipartite beetle microbiome. mBio 11, e02430–19 (2020). [PubMed: 32098813] While most highly reduced symbiont genomes show no evidence of gene acquisition, this study revealed an exception: the extracellular symbiont of the beetle *Lagria villosa* acquired beneficial genes through HGT, even while undergoing massive genome reduction.
55. Sonnenburg ED et al. Diet-induced extinctions in the gut microbiota compound over generations. Nature 529, 212–215 (2016). [PubMed: 26762459]
56. Blaser MJ Missing Microbes: How the Overuse of Antibiotics Is Fueling Our Modern Plagues. (Macmillan, 2014).
57. Daisley BA, Chmiel JA, Pitek AP, Thompson GJ & Reid G Missing microbes in bees: How systematic depletion of key symbionts erodes immunity. Trends Microbiol 28, 1010–1021 (2020). [PubMed: 32680791]
58. Moeller AH et al. Cospeciation of gut microbiota with hominids. Science 353, 380–382 (2016). [PubMed: 27463672]
59. Bourguignon T et al. Rampant host switching shaped the termite gut microbiome. Curr. Biol 28, 649–654.e2 (2018). [PubMed: 29429621]
60. Powell JE, Martinson VG, Urban-Mead K & Moran NA Routes of acquisition of the gut microbiota of the honey bee *Apis mellifera*. Appl. Environ. Microbiol 80, 7378–7387 (2014). [PubMed: 25239900]
61. Moeller AH, Suzuki TA, Phifer-Rixey M & Nachman MW Transmission modes of the mammalian gut microbiota. Science 362, 453–457 (2018). [PubMed: 30361372]
62. Yassour M et al. Strain-level analysis of mother-to-child bacterial transmission during the first few months of life. Cell Host Microbe 24, 146–154.e4 (2018). [PubMed: 30001517]
63. Kwong WK et al. Dynamic microbiome evolution in social bees. Sci. Adv 3, e1600513 (2017). [PubMed: 28435856]

64. Ohbayashi T et al. Insect's intestinal organ for symbiont sorting. Proc. Natl. Acad. Sci. U. S. A 112, E5179–E5188 (2015). [PubMed: 26324935]
65. Itoh H et al. Host–symbiont specificity determined by microbe–microbe competition in an insect gut. Proc. Natl. Acad. Sci. U. S. A 116, 22673–22682 (2019). [PubMed: 31636183]
66. Visick KL, Foster J, Doino J, McFall-Ngai M & Ruby EG *Vibrio fischeri* lux genes play an important role in colonization and development of the host light organ. J. Bacteriol 182, 4578–4586 (2000). [PubMed: 10913092]
67. Moriano-Gutierrez S et al. Critical symbiont signals drive both local and systemic changes in diel and developmental host gene expression. Proc. Natl. Acad. Sci. U. S. A 116, 7990–7999 (2019). [PubMed: 30833394]
68. Thompson CM, Tischler AH, Tarnowski DA, Mandel MJ & Visick KL Nitric oxide inhibits biofilm formation by *Vibrio fischeri* via the nitric oxide sensor HnoX. Mol. Microbiol 111, 187–203 (2019). [PubMed: 30299554]
69. Essock-Burns T, Bongrand C, Goldman WE, Ruby EG & McFall-Ngai MJ Interactions of symbiotic partners drive the development of a complex biogeography in the squid-*Vibrio* symbiosis. mBio 11, e00853–20 (2020). [PubMed: 32457244]
70. Raina J-B, Fernandez V, Lambert B, Stocker R & Seymour JR The role of microbial motility and chemotaxis in symbiosis. Nat. Rev. Microbiol 17, 284–294 (2019). [PubMed: 30923350]
71. Robinson CD et al. Experimental bacterial adaptation to the zebrafish gut reveals a primary role for immigration. PLoS Biol 16, e2006893 (2018). [PubMed: 30532251]
72. Lebov JF, Schlomann BH, Robinson CD & Bohannan BJM Phenotypic parallelism during experimental adaptation of a free-living bacterium to the zebrafish gut. mBio 11, e01519–20 (2020).
73. Erturk-Hasdemir D et al. Symbionts exploit complex signaling to educate the immune system. Proc. Natl. Acad. Sci. U. S. A 116, 26157–26166 (2019).
74. Wexler AG et al. Human gut *Bacteroides* capture vitamin B12 via cell surface-exposed lipoproteins. eLife 7, e37138 (2018). [PubMed: 30226189]
75. Putnam EE & Goodman AL B vitamin acquisition by gut commensal bacteria. PLoS Pathog 16, e1008208 (2020). [PubMed: 31971969]
76. Coyne MJ & Comstock LE Type VI secretion systems and the gut microbiota. Microbiol. Spectr 7, PSIB–0009–2018 (2019).
77. Steele MI, Kwong WK, Whiteley M & Moran NA Diversification of Type VI secretion system toxins reveals ancient antagonism among bee gut microbes. mBio 8, e01630–17 (2017). [PubMed: 29233893]
78. Speare L et al. Bacterial symbionts use a type VI secretion system to eliminate competitors in their natural host. Proc. Natl. Acad. Sci. U. S. A 115, E8528–E8537 (2018). [PubMed: 30127013]
79. Baquero F, Lanza VF, Baquero M-R, Del Campo R & Bravo-Vázquez DA Microcins in Enterobacteriaceae: Peptide antimicrobials in the eco-active intestinal chemosphere. Front. Microbiol 10, 2261 (2019). [PubMed: 31649628]
80. Frazão N, Sousa A, Lässig M & Gordo I Horizontal gene transfer overrides mutation in *Escherichia coli* colonizing the mammalian gut. Proc. Natl. Acad. Sci. U. S. A 116, 17906–17915 (2019). [PubMed: 31431529] Using experimental evolution of a target *E. coli* strain introduced to hosts with resident microbiomes, this study found that bacteriophage-mediated HGT is a strong force mediating the evolution of *E. coli* in the mouse gut.
81. Ramiro RS, Durão P, Bank C & Gordo I Low mutational load and high mutation rate variation in gut commensal bacteria. PLoS Biol 18, e3000617 (2020). [PubMed: 32155146]
82. Brockhurst MA et al. The ecology and evolution of pangenomes. Curr. Biol 29, R1094–R1103 (2019). [PubMed: 31639358]
83. Pasolli E et al. Extensive unexplored human microbiome diversity revealed by over 150,000 genomes from metagenomes spanning age, geography, and lifestyle. Cell 176, 649–662.e20 (2019). [PubMed: 30661755]
84. Vatanen T et al. Genomic variation and strain-specific functional adaptation in the human gut microbiome during early life. Nat. Microbiol 4, 470–479 (2019). [PubMed: 30559407]

85. Ludvigsen J, Porcellato D & L'Abée-Lund TM Geographically widespread honeybee-gut symbiont subgroups show locally distinct antibiotic-resistant patterns. Mol. Ecol 26, 6590–6607 (2017). [PubMed: 29087008]

86. Zheng H et al. Metabolism of toxic sugars by strains of the bee gut symbiont *Gilliamella apicola*. mBio 7, e01326–16 (2016). [PubMed: 27803186]

87. Jahn MT et al. A phage protein aids bacterial symbionts in eukaryote immune evasion. Cell Host Microbe 26, 542–550.e5 (2019). [PubMed: 31561965]

88. Wexler AG et al. Human symbionts inject and neutralize antibacterial toxins to persist in the gut. Proc. Natl. Acad. Sci. U. S. A 113, 3639–3644 (2016). [PubMed: 26957597]

89. Ross BD et al. Human gut bacteria contain acquired interbacterial defence systems. Nature 575, 224–228 (2019). [PubMed: 31666699]

90. Sonnenburg ED et al. Specificity of polysaccharide use in intestinal bacteroides species determines diet-induced microbiota alterations. Cell 141, 1241–1252 (2010). [PubMed: 20603004]

91. Fehlner-Peach H et al. Distinct polysaccharide utilization profiles of human intestinal *Prevotella copri* isolates. Cell Host Microbe 26, 680–690.e5 (2019). [PubMed: 31726030]

92. Zheng H et al. Division of labor in honey bee gut microbiota for plant polysaccharide digestion. Proc. Natl. Acad. Sci. U. S. A 116, 25909–25916 (2019). [PubMed: 31776248]

93. Hehemann J-H, Kelly AG, Pudlo NA, Martens EC & Boraston AB Bacteria of the human gut microbiome catabolize red seaweed glycans with carbohydrate-active enzyme updates from extrinsic microbes. Proc. Natl. Acad. Sci. U. S. A 109, 19786–19791 (2012). [PubMed: 23150581]

94. Kent AG, Vill AC, Shi Q, Satlin MJ & Brito IL Widespread transfer of mobile antibiotic resistance genes within individual gut microbiomes revealed through bacterial Hi-C. Nat. Commun 11, 4379 (2020). [PubMed: 32873785]

95. Foster KR & Bell T Competition, not cooperation, dominates interactions among culturable microbial species. Curr. Biol 22, 1845–1850 (2012). [PubMed: 22959348]

96. Machado D et al. Polarization of microbial communities between competitive and cooperative metabolism. Nat. Ecol. Evol 5, 195–203 (2021). [PubMed: 33398106]

97. Morris JJ, Lenski RE & Zinser ER The Black Queen Hypothesis: evolution of dependencies through adaptive gene loss. mBio 3, 300036–300012 (2012).

98. Preussger D, Giri S, Muhsal LK, Oña L & Kost C Reciprocal fitness feedbacks promote the evolution of mutualistic cooperation. Curr. Biol 30, 3580–3590.e7 (2020). [PubMed: 32707067]

99. Salem H et al. Symbiont digestive range reflects host plant breadth in herbivorous beetles. Curr. Biol 30, 2875–2886.e4 (2020). [PubMed: 32502409] Genome sequences for 13 strains of *Stammera*, the extracellular symbiont of leaf beetles, revealed that strains differ in metabolic capabilities, potentially shaping host ecological range.

100. Hansen AK & Moran NA Altered tRNA characteristics and 3' maturation in bacterial symbionts with reduced genomes. Nucleic Acids Res 40, 7870–7884 (2012). [PubMed: 22689638]

101. Van Leuven JT, Mao M, Xing DD, Bennett GM & McCutcheon JP Cicada endosymbionts have tRNAs that are correctly processed despite having genomes that do not encode all of the tRNA processing machinery. mBio 10, e01950–18 (2019). [PubMed: 31213566]

102. Melnikov SV, van den Elzen A, Stevens DL, Thoreen CC & Söll D Loss of protein synthesis quality control in host-restricted organisms. Proc. Natl. Acad. Sci. U. S. A 115, E11505–E11512 (2018). [PubMed: 30455292] This study shows that most host-restricted, small genome symbionts possess aminoacyl-tRNA synthetases with degraded editing sites, suggesting error-prone translation and demonstrating that even retained genes in eroded genomes have compromised functionality.

103. Bennett GM & Mao M Comparative genomics of a quadripartite symbiosis in a planthopper host reveals the origins and rearranged nutritional responsibilities of anciently diverged bacterial lineages. Environ. Microbiol 20, 4461–4472 (2018). [PubMed: 30047196]

104. Nakabachi A & Okamura K Diaphorin, a polyketide produced by a bacterial symbiont of the Asian citrus psyllid, kills various human cancer cells. PLoS One 14, e0218190 (2019). [PubMed: 31181122]

105. Nakabachi A, Piel J, Malenovský I & Hirose Y Comparative genomics underlines multiple roles of *Profftella*, an obligate symbiont of psyllids: providing toxins, vitamins, and carotenoids. Genome Biol. Evol 12, 1975–1987 (2020). [PubMed: 32797185]
106. Reis F et al. Bacterial symbionts support larval sap feeding and adult folivory in (semi-) aquatic reed beetles. Nat. Commun 11, 2964 (2020). [PubMed: 32528063]
107. Luan J, Sun X, Fei Z & Douglas AE Maternal inheritance of a single somatic animal cell displayed by the bacteriocyte in the whitefly *Bemisia tabaci*. Curr. Biol 28, 459–465.e3 (2018). [PubMed: 29395925]
108. Rio RVM et al. Insight into the transmission biology and species-specific functional capabilities of tsetse (Diptera: Glossinidae) obligate symbiont *Wigglesworthia*. mBio 3, e00240–11 (2012). [PubMed: 22334516]
109. Maire J et al. Spatial and morphological reorganization of endosymbiosis during metamorphosis accommodates adult metabolic requirements in a weevil. Proc. Natl. Acad. Sci. U. S. A 117, 19347–19358 (2020). [PubMed: 32723830]
110. Campbell MA et al. Genome expansion via lineage splitting and genome reduction in the cicada endosymbiont Hodgkinia. Proc. Natl. Acad. Sci. U. S. A 112, 10192–10199 (2015). [PubMed: 26286984]
111. Campbell MA, Łukasik P, Simon C & McCutcheon JP Idiosyncratic genome degradation in a bacterial endosymbiont of periodical cicadas. Curr. Biol 27, 3568–3575.e3 (2017). [PubMed: 29129532]
112. Price DRG & Wilson ACC A substrate ambiguous enzyme facilitates genome reduction in an intracellular symbiont. BMC Biol 12, 110 (2014). [PubMed: 25527092]
113. Zhang B, Leonard SP, Li Y & Moran NA Obligate bacterial endosymbionts limit thermal tolerance of insect host species. Proc. Natl. Acad. Sci. U. S. A 116, 24712–24718 (2019). [PubMed: 31740601]
114. Fan Y & Wernegreen JJ Can't take the heat: high temperature depletes bacterial endosymbionts of ants. Microb. Ecol 66, 727–733 (2013). [PubMed: 23872930]
115. Kupper M, Gupta SK, Feldhaar H & Gross R Versatile roles of the chaperonin GroEL in microorganism-insect interactions. FEMS Microbiol. Lett 353, 1–10 (2014). [PubMed: 24460534]
116. Fares MA, Ruiz-González MX, Moya A, Elena SF & Barrio E Endosymbiotic bacteria: groEL buffers against deleterious mutations. Nature 417, 398 (2002). [PubMed: 12024205]
117. Poliakov A et al. Large-scale label-free quantitative proteomics of the pea aphid-*Buchnera* symbiosis. Mol. Cell. Proteomics 10, M110.007039 (2011).
118. Husnik F et al. Horizontal gene transfer from diverse bacteria to an insect genome enables a tripartite nested mealybug symbiosis. Cell 153, 1567–1578 (2013). [PubMed: 23791183]
119. Bublitz DC et al. Peptidoglycan production by an insect-bacterial mosaic. Cell 179, 703–712 (2019). [PubMed: 31587897]
120. Nikoh N & Nakabachi A Aphids acquired symbiotic genes via lateral gene transfer. BMC Biol 7, 12 (2009). [PubMed: 19284544]
121. Mao M, Yang X & Bennett GM Evolution of host support for two ancient bacterial symbionts with differentially degraded genomes in a leafhopper host. Proc. Natl. Acad. Sci. U. S. A 115, E11691–E11700 (2018). [PubMed: 30463949]
122. Matsuura Y et al. Recurrent symbiont recruitment from fungal parasites in cicadas. Proc. Natl. Acad. Sci. U. S. A 115, E5970–E5979 (2018). [PubMed: 29891654]
123. Manzano-Marín A, Szabó G, Simon J, Horn M & Latorre A Happens in the best of subfamilies: establishment and repeated replacements of co-obligate secondary endosymbionts within Lachninae aphids. Environ. Microbiol 19, 393–408 (2017). [PubMed: 27902872]
124. Duron O et al. Tick-bacteria mutualism depends on B vitamin synthesis pathways. Curr. Biol 28, 1896–1902.e5 (2018). [PubMed: 29861133]
125. Mao M & Bennett GM Symbiont replacements reset the co-evolutionary relationship between insects and their heritable bacteria. ISME J 14, 1384–1395 (2020). [PubMed: 32076126]
126. Feng H, Edwards N & Anderson CMH Trading amino acids at the aphid–*Buchnera* symbiotic interface. Proc. Natl. Acad. Sci. U. S. A 116, 16003–16011 (2019). [PubMed: 31337682]

127. Lu H-L, Chang C-C & Wilson ACC Amino acid transporters implicated in endocytosis of *Buchnera* during symbiont transmission in the pea aphid. Evodevo 7, 24 (2016). [PubMed: 27895889]

128. Gerardo NM et al. Immunity and other defenses in pea aphids, *Acyrthosiphon pisum*. Genome Biol 11, R21 (2010). [PubMed: 20178569]

129. Gerardo NM, Hoang KL & Stoy KS Evolution of animal immunity in the light of beneficial symbioses. Philos. Trans. R. Soc. Lond. B Biol. Sci 375, 20190601 (2020). [PubMed: 32772666]

130. Chung SH, Jing X, Luo Y & Douglas AE Targeting symbiosis-related insect genes by RNAi in the pea aphid-*Buchnera* symbiosis. Insect Biochem. Mol. Biol 95, 55–63 (2018). [PubMed: 29526771]

131. Maire J et al. Weevil *pgrp-lb* prevents endosymbiont TCT dissemination and chronic host systemic immune activation. Proc. Natl. Acad. Sci. U. S. A 116, 5623–5632 (2019). [PubMed: 30819893] This study shows that hosts can evolve to limit their own immune response to beneficial symbionts: cereal weevils use a bacteriocyte-specific isoform of peptidoglycan recognition protein to cleave peptidoglycan monomers of *Sodalis* symbionts, preventing the costly activation of their immune system by their symbiont's peptidoglycan.

132. Kobiałka M, Michalik A, Walczak M, Junkiert Ł & Szklarzewicz T *Sulcia* symbiont of the leafhopper *Macrosteles laevis* (Ribaut, 1927) (Insecta, Hemiptera, Cicadellidae: Deltocephalinae) harbors *Arsenophonus* bacteria. Protoplasma 253, 903–912 (2016). [PubMed: 26188921]

133. Husnik F & McCutcheon JP Repeated replacement of an intrabacterial symbiont in the tripartite nested mealybug symbiosis. Proc. Natl. Acad. Sci. U. S. A 113, E5416–24 (2016). [PubMed: 27573819]

134. Michalik A, Jankowska W, Kot M, Gołas A & Szklarzewicz T Symbiosis in the green leafhopper, *Cicadella viridis* (Hemiptera, Cicadellidae). Association in *statu nascendi*? Arthropod Struct. Dev 43, 579–587 (2014). [PubMed: 25102427]

135. Scholz M et al. Large scale genome reconstructions illuminate *Wolbachia* evolution. Nat. Commun 11, 5235 (2020). [PubMed: 33067437]

136. Sanaei E, Charlat S & Engelstädter J *Wolbachia* host shifts: routes, mechanisms, constraints and evolutionary consequences. Biol. Rev. Camb. Philos. Soc 96, 433–453 (2020). [PubMed: 33128345]

137. Herren JK, Paredes JC, Schüpfer F & Lemaitre B Vertical transmission of a *Drosophila* endosymbiont via cooption of the yolk transport and internalization machinery. mBio 4, 00532–00512 (2013).

138. Perreau J et al. Vertical transmission at the pathogen-symbiont interface: *Serratia symbiotica* and aphids. mBio 12, e00359–21 (2021). [PubMed: 33879583]

139. Koga R, Bennett GM, Cryan JR & Moran NA Evolutionary replacement of obligate symbionts in an ancient and diverse insect lineage. Environ. Microbiol 15, 2073–2081 (2013). [PubMed: 23574391]

140. Medina Munoz M, Spencer N, Enomoto S, Dale C & Rio RVM Quorum sensing sets the stage for the establishment and vertical transmission of *Sodalis praecaptivus* in tsetse flies. PLoS Genet 16, e1008992 (2020). [PubMed: 32797092] Experiments using gene knockouts and artificial inoculations of hosts showed that the culturable proto-symbiont *Sodalis praecaptivus* can use quorum sensing to quickly attenuate its virulence and achieve vertical transmission in a novel host.

141. Hurst GDD & Frost CL Reproductive parasitism: maternally inherited symbionts in a biparental world. Cold Spring Harb. Perspect. Biol 7, a017699 (2015). [PubMed: 25934011]

142. Harumoto T, Fukatsu T & Lemaitre B Common and unique strategies of male killing evolved in two distinct *Drosophila* symbionts. Proc. Royal Soc. B 285, 20172167 (2018).

143. Harumoto T & Lemaitre B Male-killing toxin in a bacterial symbiont of *Drosophila*. Nature 557, 252–255 (2018). [PubMed: 29720654] This study determined that male-killing by a *Spiroplasma* symbiont of *Drosophila* is caused by a toxin that targets dosage compensation machinery on the male X chromosome.

144. Shropshire JD, Rosenberg R & Bordenstein SR The impacts of cytoplasmic incompatibility factor (*cifA* and *cifB*) genetic variation on phenotypes. Genetics 217, iyaa007 (2020).This

study illustrates the power of using a model host species (*Drosophila melanogaster*) in studies of unculturable symbionts, in this case *Wolbachia*. To explore cytoplasmic incompatibility phenotypes imposed by prophage genes, transgenic *Drosophila melanogaster* were engineered to express versions of *cifA* and *cifB* from related fly species, resulting in specific incompatibility outcomes for particular combinations.

145. LePage DP et al. Prophage WO genes recapitulate and enhance *Wolbachia*-induced cytoplasmic incompatibility. Nature 543, 243–247 (2017). [PubMed: 28241146]
146. Martinez J, Klasson L, Welch JJ & Jiggins FM Life and death of selfish genes: comparative genomics reveals the dynamic evolution of cytoplasmic incompatibility. Mol. Biol. Evol 38, 2–15 (2020).
147. Hill T, Unckless RL & Perlmutter JI Rapid evolution and horizontal gene transfer in the genome of a male-killing *Wolbachia*. Preprint at bioRxiv 10.1101/2020.11.16.385294 (2020).
148. Ballinger MJ, Gawryluk RMR & Perlman SJ Toxin and genome evolution in a *Drosophila* defensive symbiosis. Genome Biol. Evol 11, 253–262 (2019). [PubMed: 30576446]
149. Oliver KM, Degnan PH, Hunter MS & Moran NA Bacteriophages encode factors required for protection in a symbiotic mutualism. Science 325, 992–994 (2009). [PubMed: 19696350]
150. Brandt JW, Chevignon G, Oliver KM & Strand MR Culture of an aphid heritable symbiont demonstrates its direct role in defence against parasitoids. Proc. Biol. Sci 284, 20171925 (2017). [PubMed: 29093227]
151. Oliver KM, Degnan PH, Burke GR & Moran NA Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. Annu. Rev. Entomol 55, 247–266 (2010). [PubMed: 19728837]
152. Degnan PH, Yu Y, Sisneros N, Wing RA & Moran NA *Hamiltonella defensa*, genome evolution of protective bacterial endosymbiont from pathogenic ancestors. Proc. Natl. Acad. Sci. U. S. A 106, 9063–9068 (2009). [PubMed: 19451630]
153. Rouïl J, Jousselin E, Coeur d'acier A, Cruaud C & Manzano-Marín A The protector within: comparative genomics of APSE phages across aphids reveals rampant recombination and diverse toxin arsenals. Genome Biol. Evol 12, 878–889 (2020). [PubMed: 32386316]
154. Verster KI et al. Horizontal transfer of bacterial cytolethal distending toxin B genes to insects. Mol. Biol. Evol 36, 2105–2110 (2019). [PubMed: 31236589]
155. Perlmutter JI et al. The phage gene wmk is a candidate for male killing by a bacterial endosymbiont. PLoS Pathog 15, e1007936 (2019). [PubMed: 31504075]
156. Ren F-R et al. Pantothenate mediates the coordination of whitefly and symbiont fitness. ISME J 15, 1655–1667 (2021). [PubMed: 33432136]
157. Ren F-R et al. Biotin provisioning by horizontally transferred genes from bacteria confers animal fitness benefits. ISME J 14, 2542–2553 (2020). [PubMed: 32572143]
158. Manzano-Marín A et al. Serial horizontal transfer of vitamin-biosynthetic genes enables the establishment of new nutritional symbionts in aphids' di-symbiotic systems. ISME J 14, 259–273 (2020). [PubMed: 31624345]
159. Bomar L, Maltz M, Colston S & Graf J Directed culturing of microorganisms using metatranscriptomics. mBio 2, 00012–00011 (2011).
160. Browne HP et al. Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. Nature 533, 543–546 (2016). [PubMed: 27144353]
161. Patel V et al. Cultivation-assisted genome of *Candidatus* Fukatsuia symbiotica; the enigmatic 'X-Type' symbiont of aphids. Genome Biol. Evol 11, 3510–3522 (2019). [PubMed: 31725149]
162. Kendra CG, Keller CM, Bruna RE & Pontes MH Conjugal DNA transfer in *Sodalis glossinidius*, a maternally inherited symbiont of tsetse flies. mSphere 5, 00864–00820 (2020).
163. Keller CM, Kendra CG, Bruna RE, Craft D & Pontes MH DNA transduction in *Sodalis* species: implications for the genetic modification of uncultured endosymbionts of insects. mSphere 6, e01331–20 (2021). [PubMed: 33597173]
164. Favia G et al. Bacteria of the genus *Asaia* stably associate with *Anopheles stephensi*, an Asian malarial mosquito vector. Proc. Natl. Acad. Sci. U. S. A 104, 9047–9051 (2007). [PubMed: 17502606]

165. Nadal-Jimenez P et al. Genetic manipulation allows *in vivo* tracking of the life cycle of the son-killer symbiont, *Arsenophonus nasoniae*, and reveals patterns of host invasion, tropism and pathology. Environ. Microbiol 21, 3172–3182 (2019). [PubMed: 31237728]
166. Rubin BE et al. Targeted genome editing of bacteria within microbial communities. Preprint at bioRxiv 10.1101/2020.07.17.209189 (2020).
167. Ronda C, Chen SP, Cabral V, Yaung SJ & Wang HH Metagenomic engineering of the mammalian gut microbiome *in situ*. Nat. Methods 16, 167–170 (2019). [PubMed: 30643213]
168. Wiles TJ et al. Modernized tools for streamlined genetic manipulation and comparative study of wild and diverse Proteobacterial lineages. mBio 9, 01877–01818 (2018).
169. Leonard SP et al. Genetic engineering of bee gut microbiome bacteria with a toolkit for modular assembly of broad-host-range plasmids. ACS Synth. Biol 7, 1279–1290 (2018). [PubMed: 29608282]
170. Visick KL, Hodge-Hanson KM, Tischler AH, Bennett AK & Mastrodomenico V Tools for rapid genetic engineering of *Vibrio fischeri*. Appl. Environ. Microbiol 84, 00850–00818 (2018).
171. McLean AHC et al. Multiple phenotypes conferred by a single insect symbiont are independent. Proc. Biol. Sci 287, 20200562 (2020). [PubMed: 32546097]
172. Moran NA & Yun Y Experimental replacement of an obligate insect symbiont. Proc. Natl. Acad. Sci. U. S. A 112, 2093–2096 (2015). [PubMed: 25561531]
173. Gong J-T et al. Stable introduction of plant-virus-inhibiting *Wolbachia* into planthoppers for rice protection. Curr. Biol 30, 4837–4845.e5 (2020). [PubMed: 33035486]
174. Ross PA, Turelli M & Hoffmann AA Evolutionary ecology of *Wolbachia* releases for disease control. Annu. Rev. Genet 53, 93–116 (2019). [PubMed: 31505135]
175. Crawford JE et al. Efficient production of male *Wolbachia*-infected *Aedes aegypti* mosquitoes enables large-scale suppression of wild populations. Nat. Biotechnol 38, 482–492 (2020). [PubMed: 32265562]
176. Riglar DT et al. Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation. Nat. Biotechnol 35, 653–658 (2017). [PubMed: 28553941]
177. Isabella VM et al. Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria. Nat. Biotechnol 36, 857–864 (2018). [PubMed: 30102294]
178. Praveschotinunt P et al. Engineered *E. coli* Nissle 1917 for the delivery of matrix-tethered therapeutic domains to the gut. Nat. Commun 10, 5580 (2019). [PubMed: 31811125]
179. Wang S et al. Driving mosquito refractoriness to *Plasmodium falciparum* with engineered symbiotic bacteria. Science 357, 1399–1402 (2017). [PubMed: 28963255]
180. Leonard SP et al. Engineered symbionts activate honey bee immunity and limit pathogens. Science 367, 573–576 (2020). [PubMed: 32001655]
181. Belcaid M et al. Symbiotic organs shaped by distinct modes of genome evolution in cephalopods. Proc. Natl. Acad. Sci. U. S. A 116, 3030–3035 (2019). [PubMed: 30635418]
182. Douglas AE Housing microbial symbionts: evolutionary origins and diversification of symbiotic organs in animals. Philos. Trans. R. Soc. Lond. B Biol. Sci 375, 20190603 (2020). [PubMed: 32772661]
183. Li Y, Park H, Smith TE & Moran NA Gene family evolution in the pea aphid based on chromosome-level genome assembly. Mol. Biol. Evol 36, 2143–2156 (2019). [PubMed: 31173104]
184. Misof B et al. Phylogenomics resolves the timing and pattern of insect evolution. Science 346, 763–767 (2014). [PubMed: 25378627]

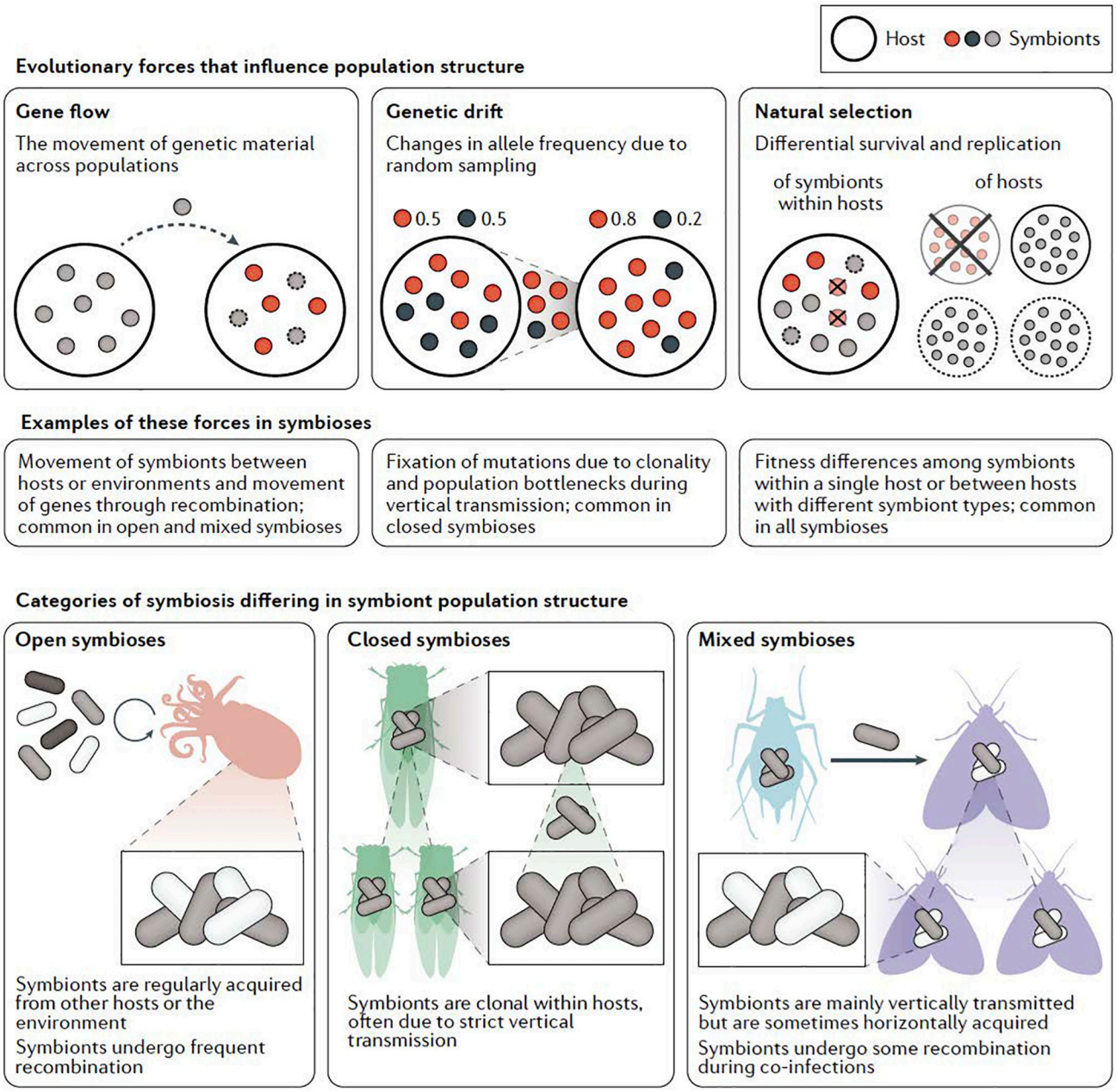

Figure 1: Symbiont genetic population structure

Genetic population structure refers to the organization of genetic variation (alleles) in a population, as a consequence of evolutionary processes including gene flow, genetic drift, and natural selection. The genetic population structure of symbionts is shaped by features of the symbiotic relationship, including the symbiont transmission mode and population bottlenecks during host colonization. These features influence the diversity of strains found within hosts, the amount of genetic recombination that symbionts undergo, and the ability of purifying selection to purge deleterious mutations that arise. Differences in genetic population structure result in different evolutionary patterns that can be categorized as open, closed, and mixed symbioses, illustrated in the lower panel. In open symbioses, such as

between the bobtail squid *Euprymna scolopes* (red) and strains of symbiotic *Aliivibrio* from the sea water, horizontal transmission and recombination are frequent. In closed symbioses, such as between *Hodgkinia cicadicola* and the cicada *Magicicada tredecim* (green), symbionts are vertically transmitted and clonal. In mixed symbioses, such as between *Hamiltonella defensa* and the aphid *Acyrthosiphon pisum* (blue), transmission is mostly vertical but occasionally horizontal across divergent hosts, as *H. defensa* is present and vertically transmitted in the whitefly *Bemisia tabaci* (purple).

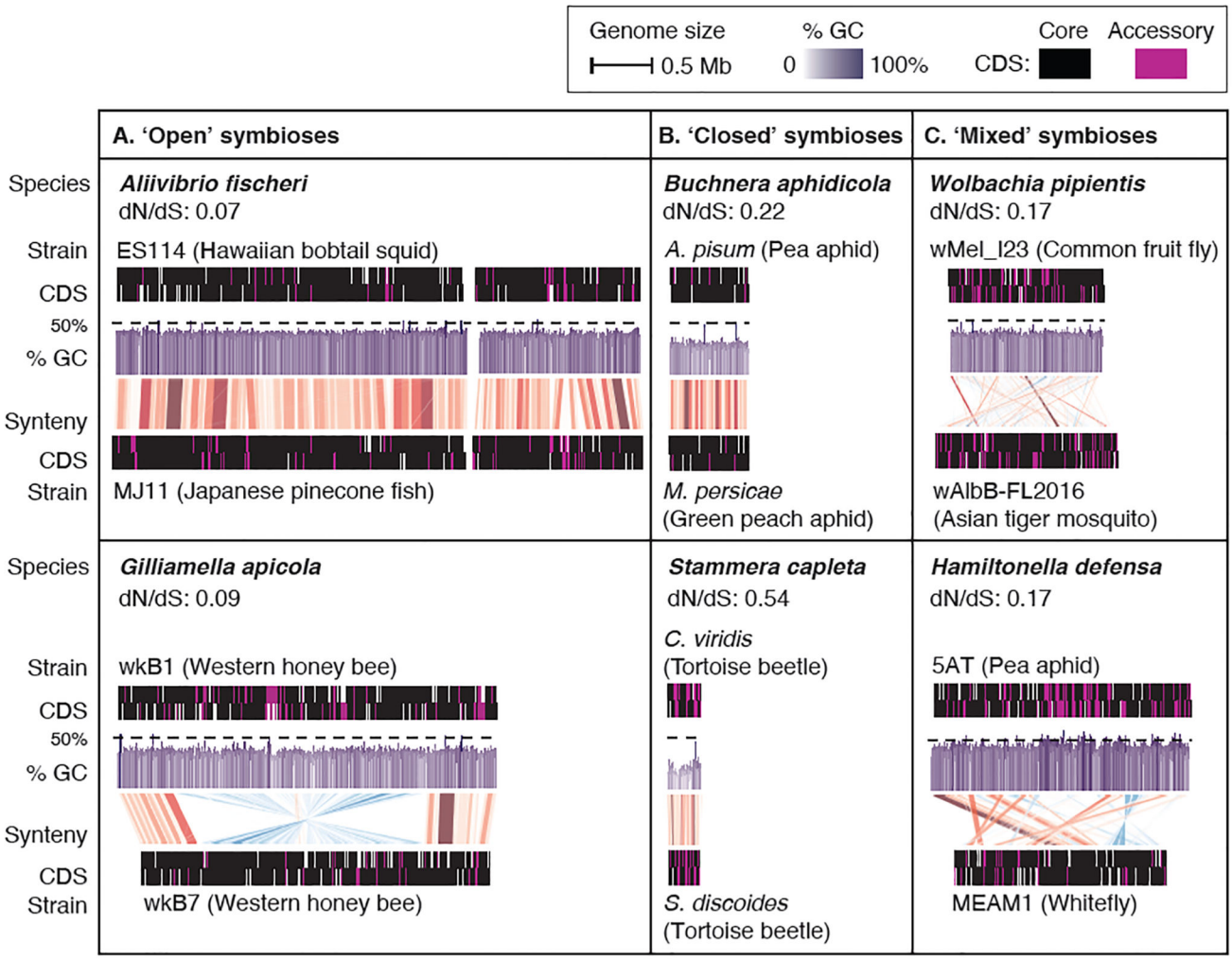

Figure 2. Genomic features of bacterial species that have evolved under open, closed, and mixed symbioses.

For each bacterial species, two strains were selected to identify core (shared) and accessory (unique) genes, to calculate a pairwise ratio of the nonsynonymous to synonymous substitution rate (d*N*/d*S*) for core genes, and to visualize intergenomic synteny. Coding sequences (CDS) are displayed for both strains, and GC content is displayed for the upper strain only (the dashed lines represent 50% GC content). Host names are given in parentheses. **A)** Symbionts in open communities retain large genomes mainly composed of protein-coding genes under strong purifying selection (d*N*/d*S* < 0.1). They possess an average or high GC content (>30%). Their genomes are mostly syntenic, although a large inversion has occurred in *G. apicola*. **B)** Symbionts in closed communities possess reduced genomes and few genes, which are under very relaxed purifying selection (d*N*/d*S* > 0.2). Their GC content is low (20 – 27% GC for *B. aphidicola* strains[42], 11–17% for *S. capleta* strains[99]). Their genomes are highly syntenic. (**C)** Symbionts in mixed communities possess genomes with varying levels of reduction, many accessory genes, and weak purifying selection (d*N*/d*S* > 0.1). They possess an average or high GC content (>30%), and their genomes possess many rearrangements and inversions.

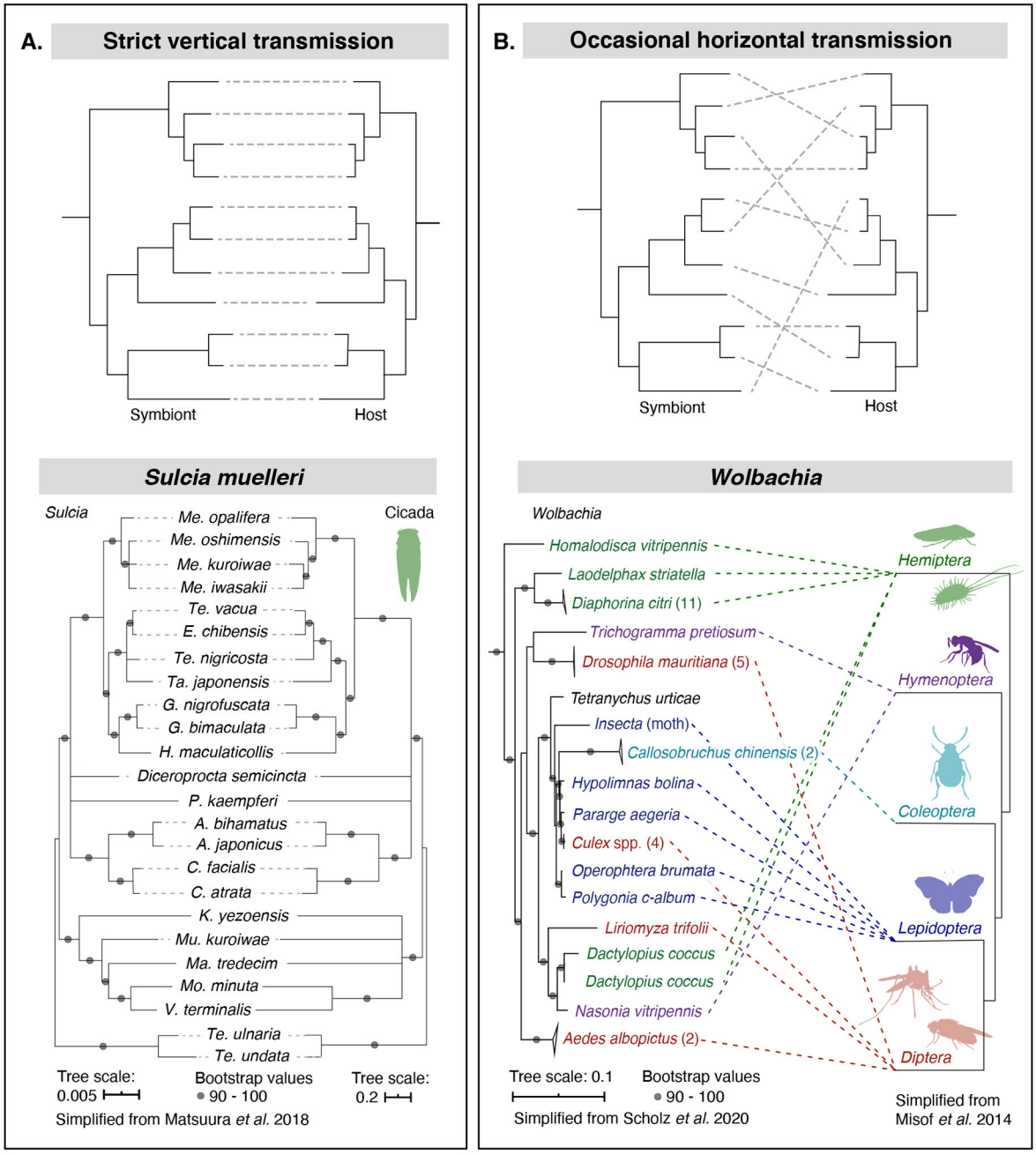

Figure 3: Symbiont phylogenetic patterns depend on the frequency of horizontal transmission.

A) Symbionts that are strictly vertically transmitted, as in closed symbioses, exhibit co-cladogenesis with their host after long timescales (top). For example, *Sulcia* has codiversified with insects in the suborder Auchenorrhyncha, including cicadas[122] (bottom). **B)** In mixed symbioses, symbionts are predominantly vertically transmitted, but occasional horizontal transmission results in mismatch of host and symbiont phylogenies over long time scales (top). For example, *Wolbachia* undergoes vertical transmission in many arthropods but shows little signal of codiversification with hosts[135] (bottom). A simplified insect phylogeny (based on data from ref[184]) is provided for reference. Part A is adapted with

permission from ref 122, National Academy of Sciences. Part B is adapted with permission from ref 135, CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

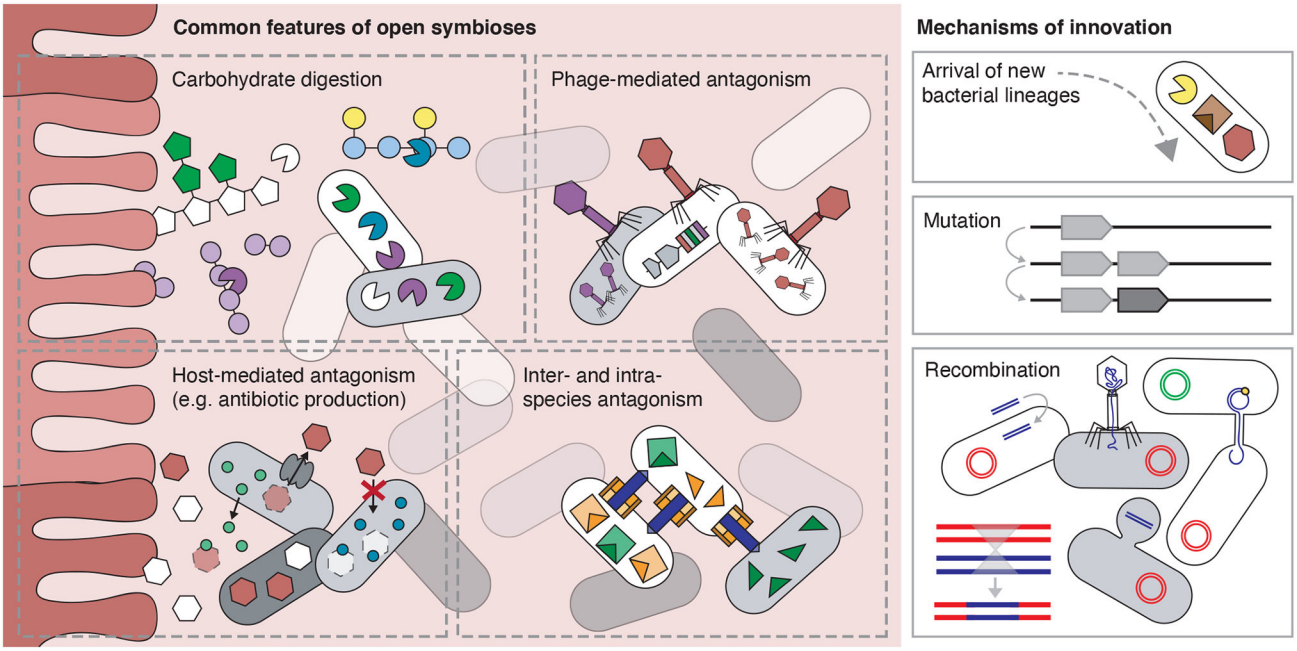

Figure 4: Common features and mechanisms of innovation in open symbiotic communities.

Bacteria in open symbiotic communities face diverse selection pressures shaped by: abiotic perturbations, such as changes to diet (top left), antibiotics (bottom left), and temperature; interactions between co-residing microbes, such as phage-mediated antagonism (top right) and T6SS-mediated antagonism (bottom right); and host social behaviors and immune response. In these communities, innovation to maintain the symbiosis, or to adapt to changing conditions, is commonly accomplished through the introduction of new strains (bottom left), through mutation (bottom centre), and through recombination (including horizontal gene transfer), which can be mediated by extracellular vesicles, transformation, transfection, or conjugation (bottom right); strong natural selection acts on the resulting variants.

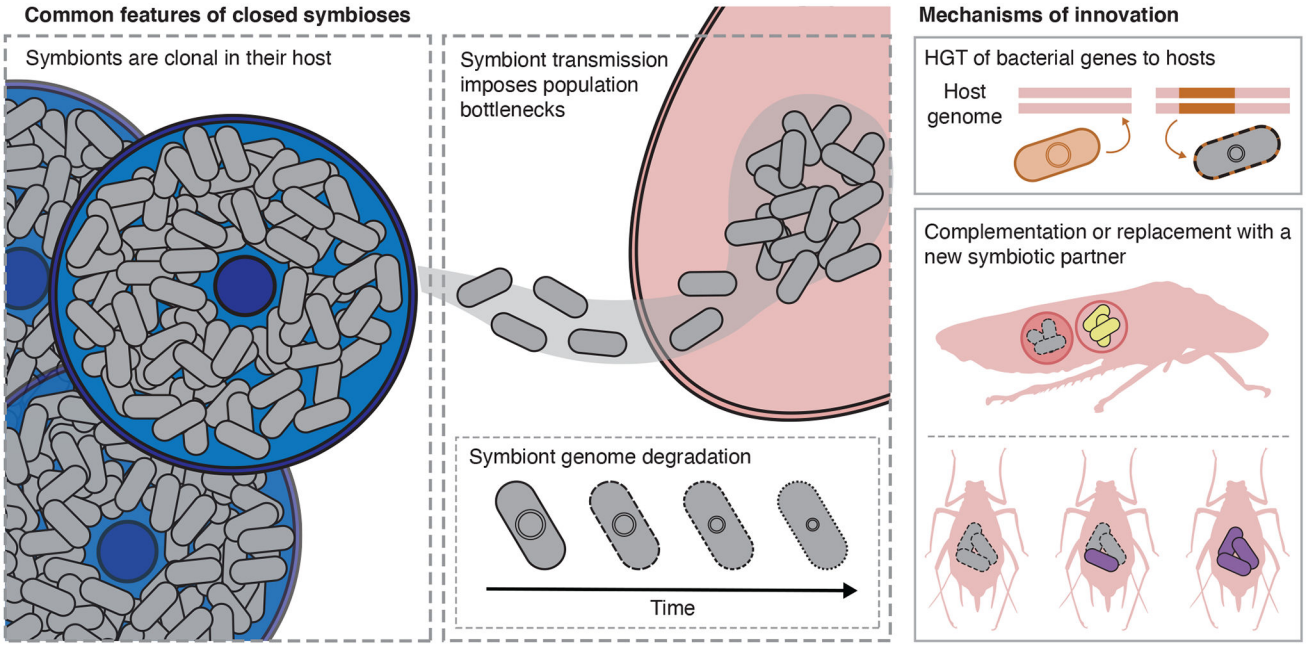

Figure 5: Common features and mechanisms of innovation in closed symbiotic communities.

Closed symbiotic communities are clonal and face population bottlenecks when transmitted to offspring. As a consequence, bacteria in closed communities accumulate deleterious mutations that they are unable to purge, and their genomes degrade with time (top). To maintain degrading symbionts, hosts innovate by acquiring bacterial genes from other bacteria, by acquiring additional symbionts, or by replacing degraded symbionts altogether (bottom). HGT, horizontal gene transfer.

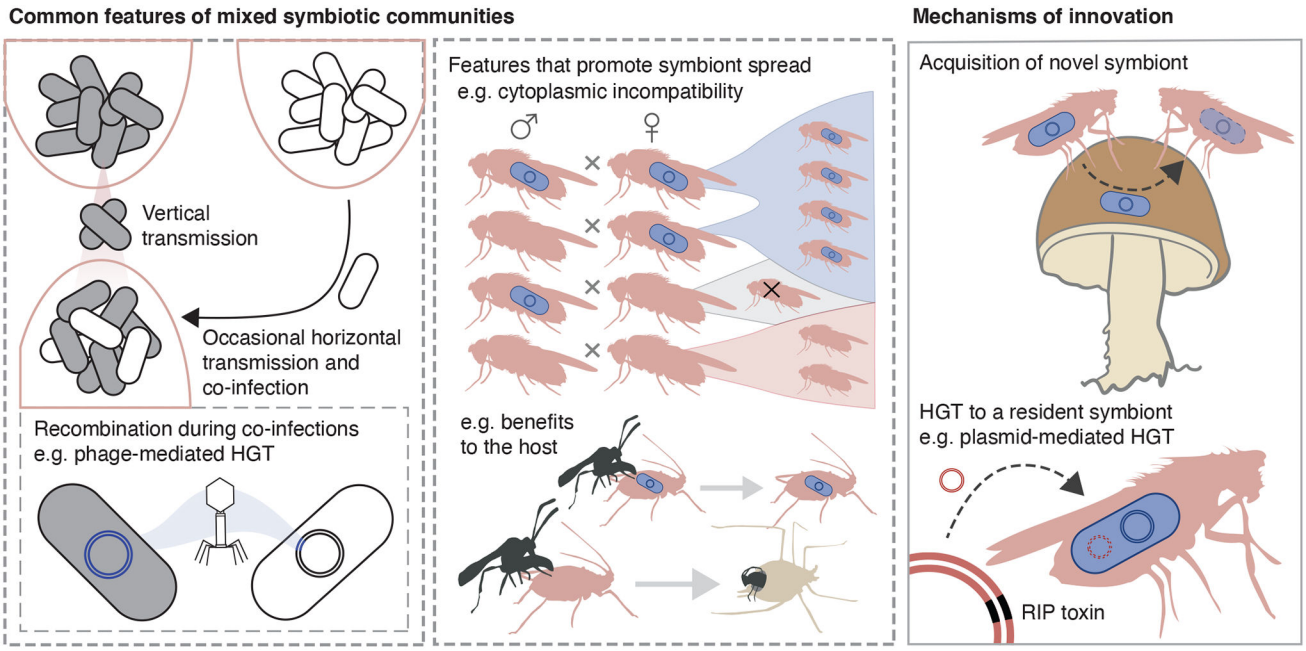

Figure 6: Common features and mechanisms of innovation in mixed symbiotic communities.

Mixed symbiotic communities are mainly clonal because of ongoing vertical transmission, but symbionts are also occasionally acquired from other hosts or the environment. Symbionts that co-infect a host can recombine and exchange genes through horizontal gene transfer (HGT), which is often mediated by phage (top left). Successful symbionts in mixed systems possess innovations that have helped them to infect new hosts and spread in host populations (top right). These innovations include the ability to manipulate host reproduction in a way that favors symbiont-bearing hosts (for example, cytoplasmic incompatibility, whereby infected males induce sterility of non-infected females), or to provide a benefit that increases host survival or reproduction (for example, by providing defense against parasitic wasps). Hosts can innovate by acquiring novel symbionts (bottom left), and symbionts are known to innovate through horizontal gene transfer (bottom right). These mechanisms of innovation are illustrated by the symbiosis between *Drosophila* flies and their defensive *Spiroplasma* symbionts[148]. RIP, ribosome-inactivating protein

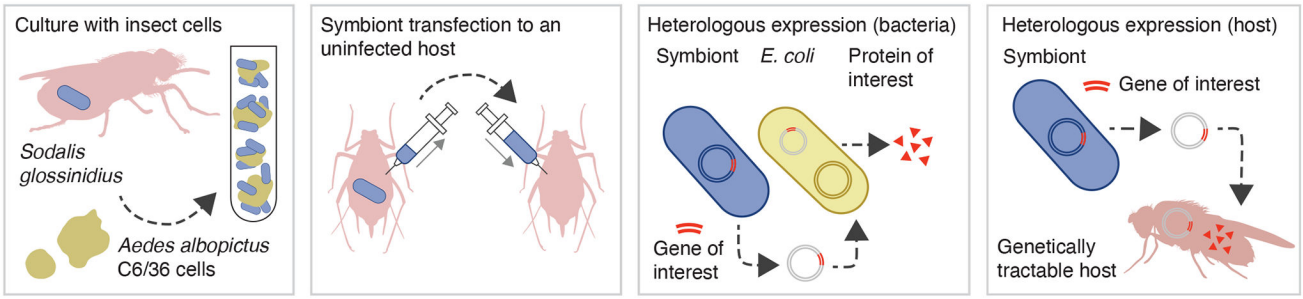

Figure 7: Commonly used tools for the study of symbiont genetics.

Symbionts that live intracellularly often possess reduced genomes and are difficult to culture or genetically engineer, limiting the study of symbiont genetics. Some common strategies have been applied to overcome these limitations. Certain symbionts can be cultured in eukaryotic cell lines, and others can be transferred from infected to uninfected hosts. Where sequencing has uncovered variation in gene content across symbiont strains, symbiont culture or symbiont transfer has been used to validate the role of certain host-beneficial genes. Lastly, symbiont gene function can be studied by heterologous expression, that is, expression of symbiont genes in genetically tractable bacteria or hosts.