Evolutionary transitions in bacterial symbiosis

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Diverse bacterial lineages form beneficial infections with eukaryotic hosts. The origins, evolution, and breakdown of these mutualisms represent important evolutionary transitions. To examine these key events, we synthesize data from diverse interactions between bacteria and eukaryote hosts. Five evolutionary transitions are investigated, including the origins of bacterial associations with eukaryotes, the origins and subsequent stable maintenance of bacterial mutualism with hosts, the capture of beneficial symbionts via the evolution of strict vertical transmission within host lineages, and the evolutionary breakdown of bacterial mutualism. Each of these transitions has occurred many times in the history of bacterial–eukaryote symbiosis. We investigate these evolutionary events across the bacterial domain and also among a focal set of well studied bacterial mutualist lineages. Subsequently, we generate a framework for examining evolutionary transitions in bacterial symbiosis and test hypotheses about the selective, ecological, and genomic forces that shape these events.

conflict | cooperation | endosymbiont | major transition | phylogeny

Ancestrally, bacteria and archaea persisted solely as free-living cells in terrestrial and aquatic habitats. Along with the evolution and diversification of animals and plants, the past 500 million years have also witnessed a massive radiation of bacteria. Bacterial lineages have evolved diverse mechanisms to gain entry and proliferate in the tissues and cells of multicellular eukaryotes (1–4), and these symbionts vary in their effect on hosts from harmful to beneficial (3, 4). Archaea have also evolved associations with hosts, but these interactions do not appear as diverse or ubiquitous. Bacterial symbioses (defined in the broad sense) include persistent, intimate associations between bacteria and other species and date back at least to the origins of eukaryotes (5). Bacterial parasites range from infectious diseases that rapidly exploit hosts before infecting new individuals, to bacteria that are transmitted vertically from host parent to offspring and manipulate host reproduction to favor their own spread (6). Parasitic bacteria have received intense focus from researchers over the last century because harmful infections represent a critical challenge to human health and economic interests. In contrast, except for a few early pioneers (7), researchers have only recently focused on the biology of bacteria that enhance host fitness: bacterial mutualists (8).

Bacterial mutualists are diverse (1–4, 9–12) and exhibit a variety of lifestyles and coevolutionary relationships with eukaryote hosts (8) (Table 1). First, beneficial bacteria vary in their degree of reliance on hosts for reproduction. Whereas some bacterial-derived organelles and endosymbionts cannot live independently of hosts, most bacterial mutualists retain extensive environmental phases and form infections that are facultative for the bacterium (8, 13, 14). Second, bacterial mutualists inhabit diverse host tissues ranging from skin, mucosa, leaves, and roots to inter- and intracellular spaces. Some bacterial mutualists inhabit specialized structures in hosts (15–26), whereas others range widely in host mucosa or other unstructured tissues (27–29) (Table 1). Finally, bacterial mutualists provide a great variety of benefits to hosts, including nutrients (15, 17, 20, 26, 27, 30), bioluminescence (14), and antibiotic production (31–33). Although bacterial mutualists by definition provide a net fitness benefit to hosts, they can also bear features that exploit hosts (8, 34–41). As we detail later, each of these variables (degree of reliance on hosts, type of host habitat, and type of benefit provided to host) can modulate evolutionary transitions in bacterial symbiosis and can explain how and why transitions occur.

Here, we investigate evolutionary transitions that have occurred in the history of bacterial mutualism. We focus on (*i*) the origins of host association in bacteria (transitions in which environmental bacteria evolve to form intimate and persistent associations with hosts irrespective of effects on host fitness), (*ii*) the origins of bacterial mutualism from other types of bacterial lifestyles, (*iii*) shifts to the stable maintenance of bacterial mutualism, (*iv*) the capture of bacterial mutualists (via the evolution of strict vertical transmission within host lineages), and (*v*) the evolutionary breakdown of bacterial mutualism. Each of these events has occurred multiple times in the evolution of bacteria. Only symbiont capture possibly constitutes a "major evolutionary transition," defined as an integrating event in which partners lose the ability to replicate independently (13). However, loss of independence often only occurs for the symbiont.

To study broad patterns and genetic drivers of transitions, we investigate phylogenomic data that span the bacterial domain (1, 4, 9–12) (Fig. 1), and to study fine scale patterns, we also analyze a focal set of bacterial mutualists (Table 1). Our domain-level data sources include a phylogeny with 350 bacterial taxa sampled from 20 phyla (9), coupled with phenotypic host-association data (1, 4, 12, 42, 43). The focal systems include beneficial symbionts chosen to represent host and bacterial diversity, breadth in symbiotic services, and variety in transmission modes. Our analysis of historical and selective scenarios that characterize transitions in bacterial symbiosis complements other work that has focused on genomic changes (1–4). The phylogeny of Wu and colleagues (9) and the review by Toft and Andersson (4) are particularly germane to this study as they provide the domain level dataset that we use to test hypotheses.

There are caveats to consider when inferring the evolutionary history of bacterial symbiosis at broad phylogenetic scales. First, is the challenge of assigning host-association traits to bacterial species. Recent work suggests that fitness benefits provided by bacteria to hosts can be context-dependent (34, 35, 44) and evolutionarily labile (8, 36, 37), potentially blurring mutualist and parasite categories. Nonetheless, although striking exceptions exist (34, 36), the majority of well studied bacterial taxa can be unambiguously categorized into host-association categories (4, 12, 45). Second is the challenge of accurately inferring past evolutionary events, which requires a robust and well sampled phylogeny. The bacterial tree we use is well supported (9), but the sampling is sparse (relative to the domain of bacteria represented) and likely biased (only se-

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Table 1. Fourteen focal bacterial-host mutualisms analyzed

Symbiont, host	Benefits provided by bacteria to host	Host localization	Transmission among hosts	Host association origins	Mutualism breakdown	Forces stabilizing bacterial mutualism
Rhizobia (e.g., 48), legumes	Nitrogen fixation (17)	Nodules (17)	Horizontal transmission (17) with free-living stages (81)	Mutualist (Fig. 1, Fig. S1)	Abandonment events (37, 48, 81)	Partner choice (41, 76, 77)
Frankia spp., actinorhizal plants	Nitrogen fixation (15)	Nodules (15)	Horizontal transmission with free-living stages (80)	Mutualist (Fig. 1) (112)	No evidence	Unknown, host localization consistent with partner choice
Pseudonocardia spp. (fungus-growing ants)	Antibiotics (31, 33)	Crypt structures on exoskeleton (19)	Vertical transmission to offspring ant colonies (31) and horizontal transmission with environmental pool (83, 84)	Mutualist (84)	Abandonment events (84)	Byproducts (see discussion), no evidence of partner choice (33)
E. persephone, tubeworm	All nutrients (21)	Lobules in host trophosome (18, 21, 25)	Horizontal with free-living stages (21)	Ambiguous (11)	No evidence	Unknown, host localization consistent with partner choice
Burkholderia spp., stinkbugs	Unknown nutrients (107)	Midgut crypts (79)	Horizontal with free-living stages (79)	Parasite (107)	Abandonment events (79)	Unknown, host localization consistent with partner choice
B. thetaiotaomicron, humans	Nutrients (30)	Crypt structures in gut (16, 24)	Horizontal transmission (16) with free-living stages (85)	Parasite (Fig. 1)	No evidence	Byproducts (*Discussion*), partner fidelity feedback (60, 61), partner choice (24)
V. fischeri, bobtail squids	Bioluminescence (14)	Deep crypts in light organ (22)	Horizontal transmission with free-living stages (22)	Parasite (Fig. 1)	Abandonment events (86)	Partner choice (57, 78)
Prochloron spp., didemnid ascidians	Photosynthates (27)	Unstructured in cloacal cavity (26, 27)	Vertical transmission via physical transfer to larvae (27). No known free living state (96)	Mutualist (113)	No evidence of mutualism breakdown	Vertical transmission promotes partner fidelity feedback
Coriobacterium glomerans, firebugs	Aid in digestion (29)	Unstructured in guts (29)	Vertical transmission via egg inoculation. Little potential for horizontal transmission or free living stages (29)	Ambiguous (29)	No evidence of mutualism breakdown	Vertical transmission promotes partner fidelity feedback
Streptomyces philanthi, beewolves	Antibiotics (32)	Lobed antennomere reservoirs in antennae (23)	Vertical transmission via brood provisioning of bacteria (97). No known free living state	Mutualist (114)	No evidence of mutualism breakdown	Vertical transmission promotes partner fidelity feedback
"Mycetocyte" bacteria, diverse insects (20)	Amino acids, Vitamins (20)	Unstructured in mycetocytes in diverse tissues (20)	Vertical transmission via host transfer to oocytes, eggs or larvae (20)	Parasite (Fig. 1)	No evidence of mutualism breakdown	Vertical transmission promotes partner fidelity feedback
Cyanobacterium spp., water-fern	Nitrogen fixation (26)	Cavities in leaves (26)	Vertical transmission via bacterial motility, no free living stage (26)	Mutualist (115)	No evidence of mutualism breakdown	Vertical transmission promotes partner fidelity feedback
Plastids, plants Mitochondria, eukaryotes	Photosynthates Metabolism	Unstructured, intracellular	Transovarial, no free living stage	Mutualist (116) Parasite (10)	No evidence of mutualism breakdown	Vertical transmission promotes partner fidelity feedback

Bacterial symbionts are indicated with genus and species when possible, and hosts are identified with common names. "Mutualist benefits" specifies the types of resources or services that the bacterial symbionts provide to their hosts. "Host localization" specifies the location that the bacteria inhabit during the majority of or key parts of their interactions with hosts and whether these locales are structured spatially. "Transmission among hosts" specifies transmission mode and presence of free-living stages are identified. "Host-association origins" specifies the inferred ancestral condition at the origin of host association in the described lineage(s). "Mutualism breakdown" specifies evidence of evolutionary transitions in bacterial lineages from mutualism to other lifestyles, with "abandonment" referring to transitions from mutualism to an environmental lifestyle. "Forces stabilizing bacterial mutualism" specifies potential forces stabilizing cooperation in a bacterial mutualist lineage, divided into the three model classes [byproduct cooperation, partner choice, and partner fidelity feedback (57)].

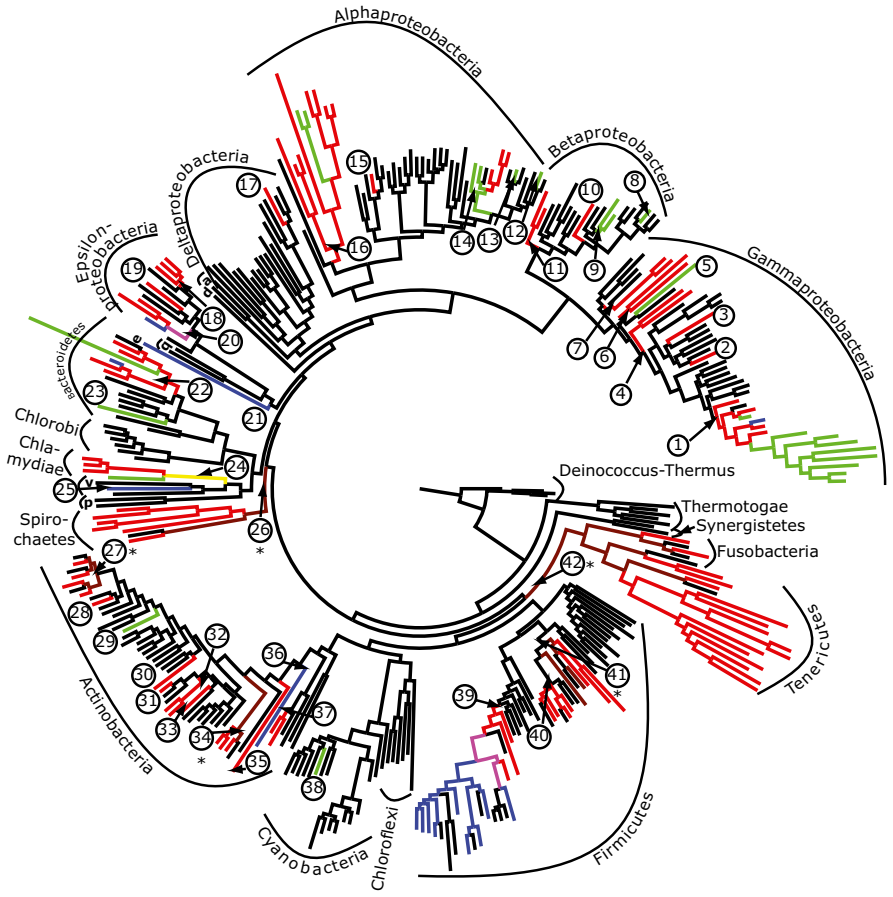

Fig. 1. Inferred evolutionary history of bacterial host association. Ancestral states are inferred on a domain-level bacterial phylogeny modified from a previous study (9). Fig. S1 and Table S1 provide taxon labels. The tree is a maximum likelihood reconstruction of a concatenated set of 31 single-copy genes from 350 bacterial species chosen to optimize phylogenetic sampling. Phyla and proteobacterial classes are labeled with their full names (e.g., Gammaproteobacteria; Firmicutes) or single-letter abbreviations (a, Acidobacteria; d, Defferribacteres; q, Aquificae; e, Elusimicrobia; v, Verrucomicrobia; p, Planctomycetes). Branch colors represent host-associated traits on the tips of the tree and inferred states on ancestral nodes (black, environmental; blue, commensal; green, mutualist; red, parasite). Host association traits were obtained from a prior review (4). We inferred a minimum of 42 origins of host-association (labeled 1–42). Origins at five nodes had equivocal parsimony reconstructions, noted with asterisks. Equivocal ancestral states are represented by blended branch colors (brown, environmental or parasite; purple, parasite or commensal; yellow, parasite or mutualist). Additional origins are equally parsimonious at these nodes and provide an upper bound for global origins at 52. (Adapted from ref. 9.)

quenced taxa are included). Finally, predictions about selective factors that drive transitions must be considered with caution, as phylogenetic comparisons often cannot distinguish evolution that predates the origins of host association from the consequences of these transitions. Our fine-scale analysis of the 14 focal symbioses serves as a complementary approach to help mitigate these challenges (Table 1).

Origins of Host Association in Bacterial Lineages

Origins of host association are transitions in which bacteria that live independently in the environment evolve to form intimate and persistent associations with hosts. To evolve host association, bacteria must be able to compete with other microbes on host surfaces, evade negative host responses, uptake novel resources on or inside the host, and ultimately gain transmission to new hosts. Considering these potential hurdles, one unanswered question is whether origins of host association are rare in bacterial lineages. Another question is whether certain bacteria taxa are more likely to evolve host association. In a phenotypic sense, this latter question addresses whether some bacteria bear preadaptations to host association.

Analyzing host association origins on a domain-level bacterial tree (4, 9) (Fig. 1; see Fig. S1 and Tables S1–S3 for taxon information), we inferred an environmental ancestral condition for the most recent common ancestor of bacteria and a minimum of 42 origins of host association across bacteria (*Methods*). An environmental ancestral condition is logical (as bacteria predate eukaryote hosts by at least 1 billion years) and is consistent with other analyses (42). Origins of host association are diversely distributed across bacteria, emerging independently in at least 11 bacterial phyla. Proteobacteria, Actinobacteria, and Firmicutes each exhibit multiple origins of host association (Table S2), whereas a few phyla such as Chlorobi, Chloroflexi, and Planctomycetes have never evolved host association (4, 9, 46).

Toft and Andersson predicted that bacterial preadaptations to host association might be ecological in nature (4), including access to mobile genes in soil and oceans and physical contact with diverse hosts, characteristics identified as common in Proteobacteria (4, 47). Although Proteobacteria exhibit 20 host-association origins, the evolutionary rate of host association origins in this lineage (estimated as origins per adjusted branch length; *Methods*) is typical for eubacteria (Table S4). Bacterial preadaptation to a host-associated lifestyle might also be genetically based, which is not mutually exclusive from ecological preadaptation. Several studies have begun to investigate genomic content changes correlated with transitions in host association, for instance by comparing phylogenetic relationships and genetic characteristics among bacterial mutualists, parasites, and related environmental species (2, 48–53). The Rhizobiales represent an excellent case study, as these α-Proteobacteria include environmental bacteria, parasites, and mutualists (2, 48). Genomic comparisons of 19 species in this lineage uncovered a relatively small subset of loci unique to the host-associated species and revealed that these loci most often originated in host-associated lineages via horizontal transfer from other host-associated bacteria (2). Other lineages that encompass parasitic and mutualistic bacteria also show a similar pattern in which host-association loci exhibit evidence of horizontal gene transfer (49–53). In summary, we found many origins of host association across bacteria and little evidence consistent with ecological or genomic predispositions to host association. The data suggest that transitions to host association might be constrained only by access to and compatibility with horizontally transferred loci that engender host-association traits (4). Nonetheless, ecological constraints to host association cannot be ruled out; the

bacterial taxa that have apparently never evolved host association might lack access to habitats with compatible hosts.

Origins of Bacterial Mutualism

Fundamental questions about the origins of bacterial mutualisms remain unresolved. Do bacterial mutualists evolve from parasitic ancestors or do they represent independent origins of host association (3, 13, 45, 54, 55)? If bacterial mutualists evolved from parasite ancestors, this predicts that transitions from parasitism to mutualism have occurred, whereas if mutualists originate separately from parasites, this predicts that mutualists have evolved directly from environmental taxa. Two scenarios have been suggested to resolve this issue. Ewald (54) introduced a detailed hypothesis for the origin of bacterial mutualism in which (*i*) an ancestral parasite infects hosts via both horizontal and vertical transmission, (*ii*) a mutation knocks out the parasite's horizontal transmission pathway, and (*iii*) subsequent vertical transmission of the bacterium selects for reduced virulence and the enhancement of mutualistic traits [as vertical transmission can link reproductive interests of symbionts and hosts (38, 39, 56, 57)]. This scenario is controversial because host-associated bacteria are thought to lack the genomic potential to easily switch from parasitism to mutualism (45). The alternative hypothesis is that bacterial mutualists evolve directly from environmental bacteria, which is also problematic because it implies that free-living ancestors exhibited traits that could offer immediate benefits to hosts (54).

We can empirically examine these alternative hypotheses by using the bacterial domain dataset (4, 9) and our focal systems (Table 1). At the domain level, many host-associated lineages are poorly sampled (Fig. 1, Fig. S1, and Table S1), so this analysis must be considered preliminary. Bacteria on the domain level tree include species classified as commensals, mutualists, and parasites (4) (Fig. S1 and Table S1). Among the 42 host-association origins we reconstructed, 32 are inferred to have originated as parasites, nine are inferred to have mutualist origins, and one origin is ambiguous (Fig. 1 and Table S1). Several mutualist taxa are nested in parasitic clades, consistent with three independent transitions from parasitism to mutualism (Fig. 1, Fig. S1, and Tables S1 and S2). It is unknown whether the evolution of vertical transmission drove these transitions because, in most lineages, the taxon sampling is poor and the order of events cannot be resolved (Table S3). Among the nine mutualist lineages that evolved directly from environmental ancestors, six are nitrogen fixing (Table S2). Consistent with Ewald's hypothesis (54), nitrogen fixation is an ancient bacterial trait (58) that can potentially offer hosts immediate benefits. However, as we observed earlier for the origins of host association, nitrogen fixation loci are also prone to horizontal transfer as parts of genome islands. This creates a scenario in which bacterial mutualists can evolve de novo from environmental ancestors via the gain of a core set of symbiosis loci (37, 59).

Among the 14 focal taxa, we can infer the host-association origins of 12 (Table 1). Three of the lineages that likely represent transitions from parasitism to mutualism are vertically transmitted (*Burkholderia* spp., "Mycetocyte" bacteria, mitochondria), consistent with the hypothesis that loss of horizontal transmission drove the origin of mutualism (54). The history of the mitochondrion is somewhat ambiguous. Although some authors have suggested that mitochondria originated from a parasitic lineage of rickettsial bacteria (45), no analysis of which we are aware has tested this hypothesis explicitly. In none of these cases can we resolve whether vertical transmission evolved before or after the transition from parasitism to mutualism (Table S3). Seven of the symbioses are inferred to have originated as mutualists directly from environmental ancestors. As described earlier, these lineages carry traits that can offer immediate benefits to hosts, including antibiotic production, nitrogen fixation, and photosynthesis (Table 1). More detailed phylogenetic analysis is needed to resolve whether these cooperative traits predate the host association, as predicted by Ewald (54). Finally, there are two symbioses that do not fit any of the aforementioned hypotheses. Both *Bacteroides thetaiotaomicron* and *Vibrio fischeri* are mutualists inferred to have evolved from parasites with no history of vertical transmission. For *B. thetaiotaomicron* (a dominant gut symbiont in humans), there is the possibility of pseudovertical transmission (60, 61). This is the hypothesis that hosts are more likely to transmit symbionts to kin, which approximates the effects of vertical transmission (60). In summary, mutualist bacteria can evolve from environmental or parasitic ancestors. Bacterial phenotypes that offer immediate benefits to hosts are thought to promote origins of mutualism in environmental bacterial lineages, but well studied cases implicate horizontal gene transfer (37, 59) as an alternative. Vertical transmission is a predicted driver of transitions from parasitism to mutualism, but there is relatively little support for vertical transmission preceding the origin of mutualism (62).

Maintenance of Bacterial Mutualism

In mutualist bacteria, it can be challenging to explain what prevents the spread of cheater mutants; symbionts that gain in fitness by exploiting hosts and giving little or nothing in return (57). Three classes of models have been proposed for the maintenance of cooperation between species—byproduct cooperation, partner fidelity feedback, and partner choice (57, 63–65)—and each of these models applies to bacterial mutualism. Byproduct cooperation occurs when the benefit provided by the symbiont to the host exists as an automatic consequence of a selfish trait, and thus byproduct cooperation carries no net cost for the symbiont (66, 67). Partner fidelity feedback exists when fitness benefits delivered from a symbiont to its host feed back as returned benefits to the symbiont, such that beneficial symbionts are rewarded and harmful symbionts experience reduced fitness (57, 64, 68). Fitness feedbacks are only expected when symbionts and hosts interact repeatedly over time, such as occurs with vertical transmission. Partner choice occurs when hosts preferentially reward beneficial symbionts and or sanction cheaters, thus producing a selective advantage for symbiont cooperation (57, 64, 69). To what degree is byproduct cooperation, partner fidelity feedback, or partner choice responsible for the maintenance of cooperative symbioses? These models can work independently or in concert with each other (57, 65); however, little empirical research has compared their prevalence.

Among our 14 focal symbioses, byproduct cooperation can mostly be ruled out, such as in Rhizobia, in which nitrogen fixation is costly and occurs only during the symbiosis (70). In contrast, we are not aware of examples in which byproduct cooperation has been demonstrated. Such scenarios are certainly possible. For instance, Actinomycete bacteria produce antibiotics on fungus-farming ants that keep the ants' fungal gardens pathogen-free (Table 1) (31). Antibiotic production is an anticompetitive function that benefits bacteria directly, whether on the surface of an ant or free in the soil, so it likely qualifies as a byproduct. Similarly, the symbiont *B. thetaiotaomicron* benefits humans by foraging and catabolizing compounds that the host cannot otherwise digest (71). The consumption of complex molecules and releasing of simpler compounds also must benefit *Bacteroides* directly. Byproduct cooperation is likely important for the origins of cooperative symbioses (57), but when interactions have been established, hosts are expected to rapidly evolve traits to promote the infection and proliferation of beneficial symbionts (65, 67). For the *B. thetaiotaomicron*–human symbiosis, these host traits might include mechanisms to bias symbiont transmission to offspring [to maximize partner fidelity (60, 61)] or mechanisms to favor beneficial strains over more selfish ones [e.g., partner choice (24)].

There is vigorous debate over the relative importance of partner fidelity feedback versus partner choice (64, 68, 72–75). Partner fidelity feedback is often equated with vertically trans-

mitted symbioses, as vertical transmission tightly correlates symbiont and host reproductive interests (57, 65). By this measure, partner fidelity is widespread across bacteria with multiple origins (Table S3) and diverse mechanisms of vertical transmission (Table 1). However, vertical transmission does not guarantee symbiont cooperation, as even rare opportunities for horizontal transfer or the potential to manipulate host reproduction can lead to parasitic bacterial phenotypes. For example, vertically transmitted parasites [such as some *Wolbachia* lineages (36)] manipulate hosts to maximize their own transmission by biasing host sex ratio toward females (they are not transmitted to males) or by inducing cytoplasmic incompatibility (6). On the contrary, most symbionts are horizontally transmitted (8, 14). Under horizontal transmission, multiple symbiont genotypes often infect hosts, and, with rare exceptions (40), partner fidelity is predicted to be weak (72, 73). Partner choice can efficiently select for symbiont cooperation under these conditions (64, 65, 69, 72, 73). Partner choice has been best demonstrated for legumes that form symbioses with nitrogen-fixing Rhizobia (41, 76, 77) and squids that form symbioses with bioluminescent *V. fischeri* (57, 78). In both examples, hosts exhibit mechanisms to reward cooperative symbionts and punish cheaters.

It can be difficult to experimentally distinguish partner-fidelity feedback from partner choice (74). However, one approach is to assess if symbionts are spatially structured within the host. The degree to which hosts can spatially separate symbiont genotypes is a key prerequisite for partner choice mechanisms (57, 69, 72, 73), but should have no bearing on partner fidelity feedback. Many hosts of horizontally transmitted bacteria have evolved specialized structures that can separate symbionts that vary in their fitness effects on the host and potentially aid in distinguishing beneficial strains from cheaters (15–26) (Table 1 and Fig. 2). In most of these examples, there is no more than a correlation between symbiotic structure on hosts and the potential for partner choice. However, these data become powerful when coupled with phylogenetic and ecological information. Kikuchi and colleagues (79) analyzed the presence and structure of midgut crypts among 124 species of stinkbugs that vary in diet as well as the presence of horizontally transmitted *Burkholderia* symbionts (Table 1 and Fig. 2). They found that (*i*) stinkbugs exhibit multiple *Burkholderia* genotype infections, a key prerequisite for partner choice; (*ii*) the *Burkholderia* symbiosis has evolved in some, but not all, of the stinkbug species that exhibit midgut crypts; (*iii*) there is no evidence that the *Burkholderia* symbiosis has evolved in stinkbug species without such crypts; and (*iv*) crypts are not strictly correlated with different feeding habits of the bugs. These data suggest that crypts—which can potentially separate beneficial from harmful symbionts (79)—are a key factor promoting stability in this bacterial mutualism. In summary, there is controversy over the relative importance of partner-fidelity feedback and partner choice as the key selective forces that maintain bacterial mutualisms (64, 68, 72–75). However, spatial separation among symbiont genotypes is a predicted indicator of partner choice (57, 69, 72, 73), and such structure is common.

Symbiont Capture

Symbiont capture occurs when bacteria that can replicate in the environment evolve to be strictly vertically transmitted within hosts and lose independent life stages. The most basal form of transmission is horizontal and likely occurs when bacteria are acquired from environmental pools (21, 80–84). In other cases of horizontal transmission, the symbiont taxa can be found in the environment (85, 86), but most transmission likely occurs among hosts (16, 60, 61, 87) with little contribution from environmental pools. Vertical transmission modes range from direct symbiont transfer within host germ lines to host behavioral mechanisms that supplement offspring with symbionts (43) (Table 1). Moreover, some bacteria cannot be easily categorized into horizontal or vertical transmission modes. For instance, some bacterial lineages are transmitted vertically, but in rare events, get horizontally

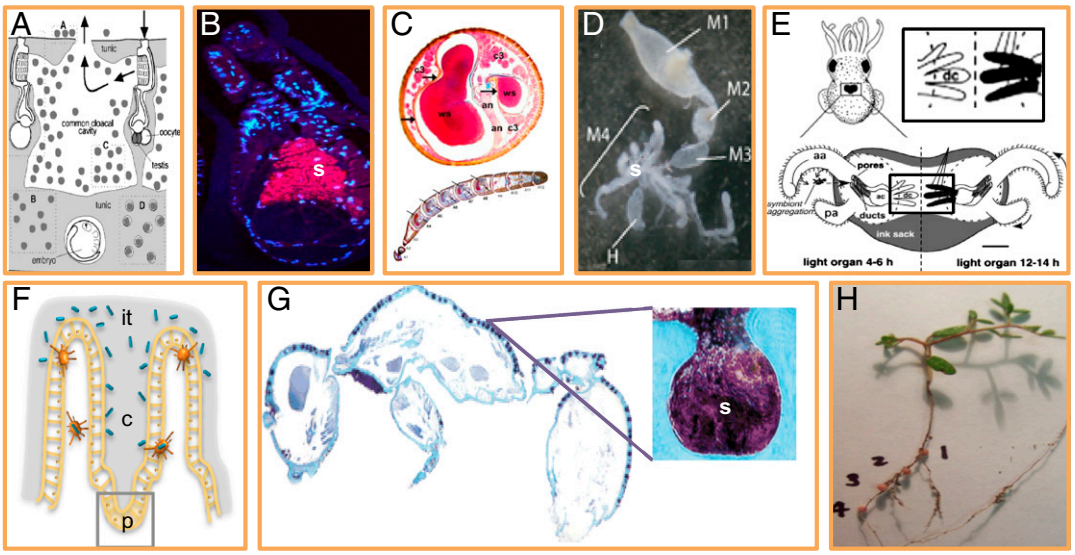

Fig. 2. Symbiont housing structures and their potential to promote spatial structure. (*A*) Host Ascidian *Diplosoma* spp. and symbiont *Prochloron* spp. unstructured in host cloacal cavity (Reprinted from ref. 28). (*B*) Host hydrothermal tubeworm *Riftia pachyptila* with symbiont *Endoriftia persephone* (s, red) unstructured in host trophosome (Reprinted from ref. 21). (*C*) Antenna of host beewolf *Philanthus triangulum* with symbiont *Streptomyces* (ws, red) housed in structured serial antennomere reservoirs (cross-section above; longitudinal section below) (Reprinted from ref. 23). (*D*). Four-chambered midgut of host stinkbug *Dimorphopterus pallipes* with symbiont *Burkholderia* spp. (s) housed in structured crypts of fourth midgut section (m4) (Reprinted from ref. 79). (*E*) Juvenile squid host *Euprymna scolopes* during colonization by symbiont *V. fischeri*, housed in structured deep crypts (dc; Adapted from ref. 22.) (*F*) Host mouse small intestine and symbiont *B. thetaiotaomicron* (blue capsules) in structured crypts of Lieberkuhn (c) based with Paneth cells (p) (Adapted from ref. 24). (*G*) Dorsal cross-section of host ant *Cyphomyrmex longiscapus* with *Actinomyces* symbionts (s) housed in structured crypts (Reprinted from ref. 19). (*H*) Host legume *Lotus strigosus* with symbiont *Bradyrhizobium japonicum* structured in four numbered nodules (photo by J. L. Sachs).

transmitted to novel hosts, likely through vectors or predation (88, 89). In most cases, captured lineages of bacteria are mutualists (our focus here), but obligate intracellular parasites such as *Wolbachia* and *Rickettsia* can also exhibit strict vertical transmission.

Symbionts with strict vertical transmission exhibit reduced effective population size and are subject to the accumulation of deleterious mutations and gene loss (90, 91), transfer of DNA to host genomes (92), and obligate reliance on the host for basic nutrient synthesis (93). Captured symbionts also experience reduced access to novel genetic material via horizontal gene transfer (4, 89), which limits the potential for novel functions to evolve and for recombination to restore function to degraded genomes. Such genome degradation tends to worsen over time (94) and ultimately cause loss of functions that are required for life outside of the host (1). Hence, vertical transmission is often an irreversible evolutionary endpoint.

An unexplored question about symbiont capture is whether host, symbiont, or joint mechanisms are responsible for these evolutionary transitions. Although the evolution of vertical transmission can be costly to symbionts, hosts experience benefits including transmitting mutualists to offspring, minimizing symbiont diversity, and reducing mixing among symbiont genotypes, all of which promote symbiont cooperation (38, 39, 57). Thus, symbiont capture should be correlated with the evolution of host mechanisms to control transmission (39). In some cases, hosts have specialized structures with no obvious function other than to transfer bacteria to offspring. Female stinkbugs bear organs on their ovipositors (95) that transfer symbionts to their eggs. The ascidian *Diplosoma similis* (27, 28, 96) exhibits a specialized "plant rake," which it extends into its cloacal cavity during spawning and thus transfers bacterial symbionts to newly spawned larvae. In many cases, vertical transmission relies on specific host behaviors, such as when females smear symbionts onto eggs, egg cases, or cocoons of offspring (20, 27, 28, 32, 95–97). However, bacterial mutualists can also promote their own vertical transmission. Among insect symbionts that inhabit mycetocyte structures within their hosts (Table 1), the bacteria sometimes migrate in the host from their mycetocyte structures to the host ovaries (20). *Wolbachia* that infect *Drosophila* use the host microtubule cytoskeleton and transport system to maximize vertical transmission (98). Moreover, the bacterial symbiont of the water fern *Azolla filiculoides* differentiates into a motile form and actively moves from adult plant leaves to infect the sporocarp of offspring plants (26). In all the examples in which the symbiont bears mechanisms to promote vertical transmission, there is no free-living existence and no potential for horizontal transfer (Table 1). Not surprisingly, when vertical transmission is the only mechanism to invade new hosts, symbiont traits are selected to enhance its efficiency. In summary, symbiont capture within host lineages involves a suite of deleterious effects that degrade symbiont genomes while providing benefits to hosts. As predicted by theory, the evolution of symbiont capture appears to be mostly driven by host mechanisms, but only a handful of bacterial–host interactions have been studied in detail (43).

Breakdown of Symbiosis

There is debate about the evolutionary robustness of mutualisms, of which beneficial microbe–host interactions are a subset. Mutualist populations have been predicted to be prone to extinction (99), the spread of cheater mutants (63, 64), and reversions to free-living existence (99–101), but other research predicts that mutualisms are robust to these challenges (102–104). Evolutionary transitions that result in the loss of mutualistic traits (105) can be divided into transitions from mutualism to parasitism and transitions from mutualism to free-living status (i.e., abandonment of mutualism). Ancient bacterial mutualisms (5, 26, 90, 106) serve as empirical examples of long-term robustness, but it is unknown whether such stability is common.

To what degree does mutualism breakdown occur in bacteria? We can investigate the evolutionary stability of bacterial mutualism by using the domain-wide phylogeny (4, 9) (Fig. 1) and our focal symbioses (Table 1). The domain-wide data can be considered only preliminary because of the paucity of dense taxon sampling. We could only infer two evolutionary transitions from mutualism to other lifestyles: one transition from mutualism to parasitism and one abandonment of mutualism. Nonetheless, this is a surprising paucity of transitions considering that we inferred 72 evolutionary transitions on the tree (Figs. 1 and 3).

Among the 14 focal systems, there is evidence of mutualism breakdown in four, all of which involve transitions from mutualism to free-living status in symbionts with extensive free-living stages (Table 1). Two particularly dynamic examples of mutualism breakdown have been uncovered in symbionts of ants (84) and stinkbugs (79, 107). In the case of the ants, the symbionts are antibiotic-producing Actinobacteria that live in cuticular crypts supported by specialized exocrine glands (19). Lineages of these Actinobacteria have likely undergone multiple transitions between host-associated and environmental status based on the intermixing of symbiotic and environmental genotypes on a population-level phylogeny (84). Similarly, a phylogeny of the *Burkholderia* bug symbionts encompasses many environmental isolates, consistent with multiple transitions from symbiotic to environmental status (79). Evidence for abandonment of symbiosis has also been found among rhizobial lineages, some of which are related to plant and mammal parasites as well as environmental bacterial species (48), suggesting the potential for multiple transitions among mutualism, parasitism, and environmental lifestyles (70) likely driven by horizontal transfer events of symbiosis loci (108). More focused analyses have inferred multiple events of evolutionary abandonment of mutualism within *Bradyrhizobium* populations (37, 81), but found no evidence of transitions from mutualism to parasitism (37). In *Bradyrhizobium*, the abandonment of mutualism appears to be driven by degradation or wholesale loss of symbiosis loci encoded on a genome island (37). Finally, there is evidence of abandonment of mutualism within lineages of beneficial *V. fischeri*, with at least three evolutionary transitions from mutualism to environmental status (86) (Table 1). In summary, among different lifestyles that bacteria can exhibit, mutualism with hosts appears to be evolutionary stable with few transitions to other lifestyles. We found transitions from mutualism to free-living status, but virtually no evidence of transitions from mutualism to parasitism.

Discussion

The evolutionary history of bacterial mutualism is rich and ancient. The origin of host association appears to be a readily surmountable step for bacteria. The commonness and near universality of this transition suggests that it is selectively advantageous and might be rarely affected by ecology. The evolution of bacterial mutualism is also common and phylogenetically diverse, and can occur via

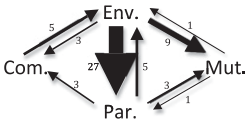

Fig. 3. Path diagram of evolutionary transitions among bacterial host-association types. Transitions among four bacterial host-association types inferred in the tree by Wu and colleagues (9) using lifestyle data from Toft and Anderson (4). Com., commensal; Env., environmental; Mut., mutualist; Par., parasite. Thirteen transitions were undetermined on the tree as a result of ambiguity. There were zero transitions between mutualism and commensalism and zero transitions from commensalism to parasitism. Arrow sizes are scaled to the number of transitions between host-association types.

multiple routes. Bacterial mutualism most often appears to emerge from environmental ancestors. This can occur because the ancestral bacteria bear key traits (that can immediately benefit hosts) or by horizontal gene transfer of symbiosis loci (37, 59), but neither mechanism is well understood. Bacterial mutualism can also arise from parasitic ancestors. It has been predicted that transitions from parasitism to mutualism are promoted by the evolution of vertical transmission (54); however, more detailed work is needed to test this hypothesis. When bacterial mutualism has evolved, it can be stabilized via several selective mechanisms (57). Partner choice, concomitant with the ability of hosts to spatially structure bacterial genotypes, is likely the dominant force maintaining bacterial mutualism.

Bacterial symbiosis first evolved with horizontal transmission, and several bacterial lineages have subsequently evolved strict vertical transmission. Some of the most ancient cases of bacterial mutualism exhibit vertical transmission, so this transition can promote the evolutionary stability of symbioses. We hypothesize that transitions from horizontal to obligate vertical transmission are host driven, as hosts (but not symbionts) most benefit from these transitions. Finally, evolutionary losses of bacterial mutualism are rare compared with other transitions in bacterial symbiosis. Evolutionary reversions from mutualism to environmental status occur in some bacterial lineages, potentially driven by the degradation or deletion of genes that encode symbiotic traits (37). In contrast, there is virtually no evidence in the phylogenetic record of transitions from mutualism to parasitism, thus refuting theory that predicts that mutualisms are vulnerable to fixation of cheater mutants (57, 63, 64). The lack of transitions from mutualism to parasitism suggests that (*i*) bacterial mutualisms are evolutionarily robust or (*ii*) transitions from mutualism to parasitism are themselves unstable [and lead to extinctions or other stable states (105)].

Methods

We analyzed evolutionary transitions on a published 350-species bacterial phylogeny reconstructed by using a concatenated alignment of 31 proteins with maximum likelihood [PhyML (109)] and an AMPHORA pipeline (9, 110) (Fig. 1, Table 1, Fig. S1, and Tables S1–S4). Host-associated phenotypes were assigned based on a recent review (4) that included host-association classifications of parasitic, mutualistic, commensal, or no interaction. We divided classifications into two characters: (*i*) association (host-associated or environmental) and (*ii*) type of host interaction (parasitic, mutualistic, commensal). Ancestral states were inferred by using parsimony [Mesquite 2.74 (111)]. When two equally parsimonious ancestral state reconstructions were found, we noted the ambiguity and listed a minimum estimate of transitions (Fig. 1).

To compare the relative frequencies of host-association origins among different bacterial lineages, we estimated the rate of origins over evolutionary time for each phylum and the complete tree (Table S4). Rates were calculated by dividing the total number of origins of host association in a lineage by an adjusted sum of the taxon's branch length. The adjusted sum included only branches on which transitions from an environmental lifestyle to host association could occur (i.e., summed branch length of the taxon minus host-associated descendant branches of previously accounted origins and individual branches on which host association has been lost). The unit of branch length is the expected number of amino acid substitutions per site.

For focal symbiont taxa, we analyzed phylogenies containing the lineages of interest to assess whether host association originated from parasitic ancestors or free-living ancestors and to search for evidence of mutualism breakdown. Ancestral states for symbiotic lineage and evidence of mutualism breakdown were inferred by using parsimony on the available phylogenies (10, 11, 29, 37, 48, 53, 79, 81, 84, 86, 107, 112–116).

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