

New paradigms for the evolution of beneficial infections

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A longstanding paradigm predicts that microbial parasites and mutualists exhibit disparate evolutionary patterns. Parasites are predicted to promote arms races with hosts, rapid evolution and sexual recombination. By contrast, mutualists have been linked with beneficial coadaptation, evolutionary stasis and asexuality. In this review we discuss the recent surge of molecular data on microbes that are being used to test and reshape these ideas. New analyses reveal that beneficial microbes often share mechanisms of infection and defense with parasites, and can also exhibit rapid evolution and extensive genetic exchange. To explain these patterns, new paradigms must take into account the varied population biology of beneficial microbes, their potential conflicts with hosts, and the mosaic nature of genome evolution that requires locus-based tests to analyze the genetics of host adaptation.

Classical models for the evolution of beneficial versus harmful infections

All known animal and plant species are infected by microbial symbionts that range in effect from harmful to beneficial. Microbial parasites have received intense interest from researchers over the past century because these harmful infections represent a key challenge to agriculture and human health. Only in the past few decades has research focused on microbes that enhance host fitness. These microbial mutualists are now known to be phylogenetically diverse [1–14], ecologically ubiquitous and central to host evolution [15,16] (Box 1). Among microbial mutualists some of the best-studied lineages are endosymbionts and organelles: bacteria that have evolved to live within host cells, are vertically transmitted and have often lost independence [9,17–21]. However, most beneficial microbes, including bacteria, fungi and unicellular algae, retain extensive environmental phases and form infections that are facultative for the microbe [5,11,16]. A longstanding paradigm has been that microbial mutualists and parasites evolve in fundamentally different ways. Here, we review and reshape these ideas in light of new theory and data, and describe new paradigms that are emerging in the field of microbial mutualism.

Classically, theoreticians have proposed opposing models for the evolution of parasitic versus mutualistic infections. Theory and empirical support are better developed for the study of microbial parasitism, so we discuss this first. Host–parasite evolution is typically modeled as an

arms race in which each partner maximizes fitness by evolving traits that reduce the fitness of the other. The common framework for this ‘Antagonistic arms race’ between hosts and parasites makes three predictions concerning the infecting microbes. Firstly, hosts selectively favor rare genotypes in their associated parasite populations by evolving defenses against commonly encountered strains (negative frequency-dependent selection; [22,23]). Secondly, rapid evolution is favored because novel microbe genotypes are more likely to escape the negative consequences of hosts adapting to them [22–24]. Finally, the Red Queen hypothesis [25] predicts that sexual recombination is selectively advantageous in interspecific arms races because sex generates offspring with novel genotype combinations that can escape host defenses [22–24]. These latter two predictions, regarding rapid evolution and sex, were originally made only for hosts (because the microbes exhibit an evolutionary rate advantage over their hosts in most cases) but are now applied to microbial parasites as well [24] (Box 2).

Empirical work largely supports this classic tripartite framework for the evolution of microbial parasites. Negative frequency-dependent selection appears as a feature of

Glossary

‘Antagonistic arms race’ models: a framework of predictions developed for host–parasite evolution that can also potentially apply to the evolution of beneficial infections [35,36]. Because symbionts and hosts experience conflicts of interest over resource exchange [15,40], transmission [43], and possibly sex [35], each partner is predicted to evolve traits to maximize its own fitness gains at the expense of the other (see Box 3 for predictions).

Endosymbiont: microbes that live within the cells or body of hosts are vertically transmitted and often cannot survive outside of the host.

Microbial mutualist: a microbe that interacts closely with a host and significantly enhances the host’s fitness.

Microbial parasite: a microbe that interacts closely with a host and significantly reduces the host’s fitness.

‘Mutualistic environment’ models: a framework of predictions introduced by Law and Lewis [31] and elaborated by others. It predicts that hosts and beneficial symbionts should evolve to ‘benefit the association’. Thus, traits that maximize the coadaptation and evolutionary stability of the interaction are selected (see Box 3 for predictions).

Positive selection: directional selection in which new genetic variants are advantageous and spread through a population; a pattern of molecular evolution often thought to be driven by inter-specific conflict.

Proteobacteria: a phylum of Gram-negative bacteria that includes many host-associated lineages. The class α -proteobacteria includes plant mutualists (*Rhizobium*) as well as parasites of both animals (*Rickettsia*) and plants (*Agrobacterium*).

Secretion systems (type III and type IV): membrane-associated protein complexes that inject protein (type III) or nucleo-protein (type IV) effector molecules into hosts, usually associated with toxicity.

Symbiont: host-associated microbes. Hosts can be animals, plants, fungi or protists, whereas symbionts can be bacteria, fungi, algae, or viruses that are beneficial, harmful or neutral in their fitness effect on hosts.

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Box 1. Diverse lifestyles of beneficial microbes

Diversity in the lifecycles and population biology of beneficial microbes correlate with variation in dependence on hosts, co-cladogenesis with host lineages and microbe genome size. **Eight beneficial host-symbiont systems are considered (a-h) in the figure below.** Black arrows indicate microbe transmission among hosts and the environment. Phylogenies of microbes (green) and hosts (black) indicate loss of the association within the microbe lineage (red branches) and degree of co-cladogenesis (dotted lines connect associated microbes and hosts), and arrows show whether genomes are often expanded or reduced in size in response to host association. **Systems and references:** (a) Legumes and nitrogen fixing rhizobia: symbiotic lifecycle [39]; host dependence, phylogenetic stability [5]; genome size [16,48]. (b) Attine ants and antibiotic-producing actinobacteria: symbiotic lifecycle, host

dependence, phylogenetic stability [11]; genome size [7]. (c) Cnidaria and zooxanthellae: symbiotic lifecycle, host dependence [12,74]; phylogenetic stability [72]; genome size [49]. (d) Humans and gut floral *Bacteroides* spp: symbiotic lifecycle [6]; genome size [50]; phylogenetic stability [1]; host dependence (no data). (e) Beewolves and antibiotic producing *Streptomyces*: symbiotic lifecycle, host dependence, phylogenetic stability [8]; genome size (in process). (f) Ascidians and photosynthetic *Prochloron*: symbiotic lifecycle, host dependence, phylogenetic stability [16]; genome size (in process). (g) Plants and organellar plastids: symbiotic lifecycle, host dependence, phylogenetic stability, genome size [9]. (h) Aphids and endosymbiotic *Buchnera*: symbiotic lifecycle, host dependence, genome size [4]; phylogenetic stability [38] (Figure 1).

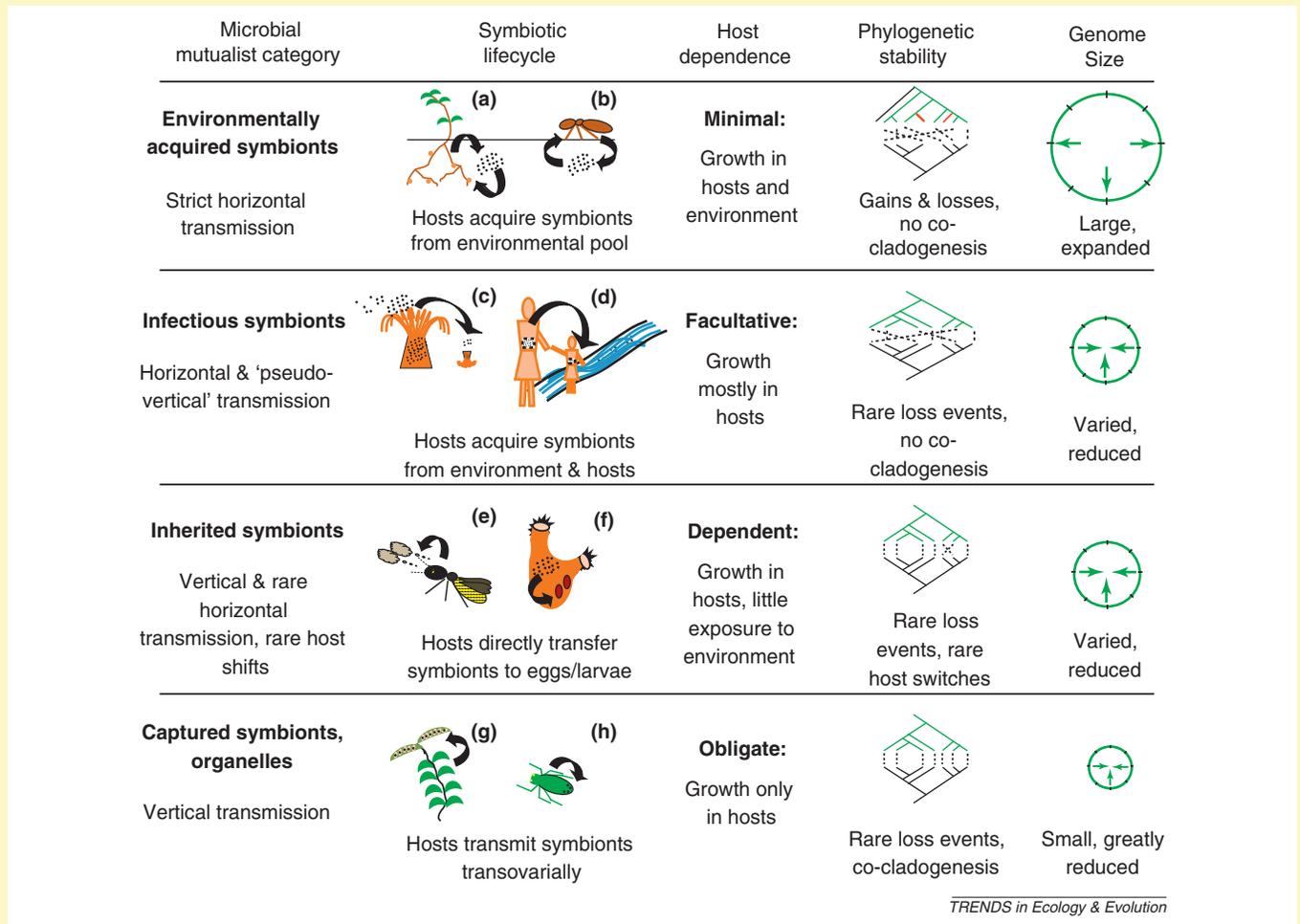


Figure 1. Microbial mutualist categories that vary in key lifestyle and evolutionary parameters.

host-parasite systems in diverse animals [26,27] and plants [28]. Evidence of rapid evolution driven by host-parasite conflict is also compelling; parasite loci that counteract host defenses often represent the fastest-evolving regions of parasite genomes [29]. Finally, evidence that parasitism can favor the evolution of microbial sex comes from highly successful parasite species that often exhibit high rates of recombination [30].

A traditional view of mutualistic infections has been that both microbes and hosts benefit by enhancing the other's fitness [31–33]. In contrast to arms race dynamics, these 'Mutualistic environment' models (Box 2), introduced by Law and Lewis [31,32], assume that hosts evolve to

favor common phenotypes of their beneficial microbes (positive frequency-dependent selection; [31–33]) and thus predict evolutionary stasis and asexuality in the microbes [31,32]. Specifically, the microbes are predicted to have lower evolutionary rates and prevalence of sex than their hosts because beneficial microbes often replicate within their hosts, whereas hosts must also interact with the environment [31,32]. More recent theory also predicts that selection might favor more slowly evolving strains of microbial mutualists. The Red King model predicts that a more slowly evolving mutualist partner will gain a disproportionate share of benefits by being evolutionarily constrained in the interaction, and thus limiting fitness gains

Box 2. Alternative frameworks for the evolution of host-associated microbes

Framework	Predictions: evolution-genetic patterns
<p>'Antagonistic arms race'</p> <p>Framework for host–parasite evolution that can potentially apply to beneficial infections: Models predict that mutualists are selected to maximize benefits and minimize costs in interspecific interactions [15,37,40], hence conflicts of interest can drive arms race dynamics similar to host–parasite evolution [35,36].</p>	<p>Negative frequency-dependent selection: hosts adapt to (and thus select <i>against</i>) the most frequent genotypes of their associated microbes. Rare genotypes of microbes are more likely to escape host defense mechanisms [22,23].</p> <p>Rapid evolution: novel genotypes of infecting microbes are more likely to escape host defense mechanisms. Increased evolutionary rate is favored as a consequence of co-evolutionary conflict [22–24].</p> <p>Sexual recombination: sex serves as a means for microbes to generate progeny with novel genotype combinations to escape host defense mechanisms [22–24].</p>
<p>'Mutualistic environment'</p> <p>Framework introduced by Law and Lewis and elaborated by others: predicts that mutualist microbes and hosts evolve to 'benefit the association' with each other [31,32].</p>	<p>Positive frequency-dependent selection: hosts adapt to (and thus select <i>for</i>) the most frequent genotypes of their associated microbes. Common microbe genotypes are more likely to find compatible, beneficial hosts than novel or rare genotypes [31–33].</p> <p>Evolutionary stasis: novel microbe mutants are selected against in beneficial populations associated with hosts because new microbe genotypes are less likely to be coadapted to common host genotypes [31,32].</p> <p>Evolution of asexuality: novel genotypes created by sexual recombination are selected against in beneficial microbial populations because new microbe recombinants are less likely to be coadapted to common host genotypes [31,32].</p>

to the more rapidly evolving partner [34]. However, because this model is based on conflict between partners it does not fit in with the 'Mutualistic environment' framework.

Law and Lewis did not test their assumption of positive frequency-dependent selection directly but provided empirical support for their subsequent predictions by comparing taxonomic diversity (a proxy for evolutionary rate) and occurrence of sex between microbial partners and their respective host lineages. They found that hosts greatly outnumbered their microbes in terms of taxonomic diversity, and they identified sexual reproduction (including meiosis, conjugation, transduction and transformation) much more often in hosts than in their respective beneficial microbes [31,32], both in support of their model. Yet, by relying mostly on phenotypic data (molecular data were still relatively sparse in the early 1980's), Law and Lewis introduced biases against the discovery of microbial diversity and sexual recombination. The recent explosion of molecular data that we discuss below provides a more nuanced view of the evolution of beneficial microbes, and in particular reveals that they can share many characteristics with microbial parasites.

Blurring lines between mutualism and parasitism

In the past decade, research has blurred the theoretical divide between mutualism and parasitism. By modeling mutualisms as reciprocally exploitative interactions (e.g. [34–39]), some theoreticians have challenged the notion that mutualists evolve in a 'Mutualistic environment' in which each partner invariably maximizes the other's fitness (e.g. [31,32]). This emerging paradigm predicts that mutualists are selected to minimize costly contributions to partners while maximizing their own fitness gains from the interaction [15,37,40]. These predictions resemble the 'Antagonis-

tic arms race' models except that both partners are selected to exploit each other while minimizing the exploitation they experience [15,35,36,39,41,42] (Box 2). For instance, in mutualistic symbioses, conflicts of interest can occur over resource exchange [15,40], symbiont transmission among hosts [43,44] and possibly sex [35]. Such conflicts can select for cheaters: individuals that exploit the interaction without providing any benefits [37,38,40,42,44], not unlike parasites. Recent empirical studies have indicated that both environmental [45,46] and evolutionary changes [42,44,47] can alter symbiotic benefit, potentially driving rapid shifts in the microbe between mutualism and parasitism [38]. Finally, molecular data are challenging the perceived evolutionary genetic differences between microbial mutualists and parasites, which is our focus here. We first review genomic analyses that reveal many genetic similarities between microbial mutualists and parasites. Subsequently, we review data to investigate the three key predictions of the 'Mutualistic environment' framework: positive frequency-dependent selection, evolutionary stasis and the evolution of asexuality. In the concluding section, we propose new paradigms for the study of microbial mutualism.

Genomic data: homologous systems in microbial parasites and mutualists

Microbial mutualists exhibit diverse transmission modes and varying degrees of reliance on hosts, and these traits are often associated with variation in key evolutionary genetic patterns, including the degree of co-cladogenesis between microbes and hosts and evolution of microbial genome size [9,48–50] (Box 1). Nonetheless, given the dramatically different effects that microbial mutualists and parasites can have on host fitness, relatively few consistent differences can be found in the content of their

Box 3. Testing the key predictions of the ‘Mutualistic environment’ framework

Predictions	Empirical datasets and references
Positive frequency-dependent selection on beneficial microbes	<p>Evolution of host loci that respond to infections: Mixed evidence: plant hosts bear fewer resistance loci and less polymorphism in resistance loci to beneficial microbes (relative to harmful microbes [33]) but host loci that modulate mutualistic infections exhibit signatures of positive selection [41,86].</p> <p>Spatial stability of interactions: Mixed evidence: interactions between legume hosts and rhizobial symbionts exhibit spatial stability of interactions [33] but several studies of other mutualistic associations have failed to find similar patterns [59].</p>
Evolutionary stasis in beneficial microbes	<p>Taxonomic diversity in microbial partners relative to hosts: Largely inconsistent with stasis: many symbiont lineages exhibit similar or greater taxonomic diversity than their hosts [2,3,62,63,72] but lichen photobionts represent an exception to this pattern [65,66].</p> <p>Evolutionary rate of vertically transmitted endosymbionts: Inconsistent with stasis: vertically transmitted bacteria exhibit increased substitution rates compared to free-living relatives but this is probably due to drift and not host adaptation [4,17–21,67,68].</p> <p>Evolutionary rate in horizontally transmitted symbionts: Consistent with stasis: the evolutionary rate of some environmentally acquired beneficial microbes is not significantly different from free-living relatives [72], whereas other taxa exhibit reduced evolutionary rate compared to free-living relatives [18,73].</p> <p>Molecular evolution of host-specific loci in symbionts: Mixed evidence: host-association loci in mutualistic microbes seem to be more constrained than homologs in pathogens [75] but nonetheless can exhibit evidence of positive selection [54,76,77].</p>
Evolution of asexuality in beneficial microbes	<p>Evidence of recombination and horizontal gene transmission: Largely inconsistent with asexuality: diverse beneficial microbes exhibit extensive recombination [49,81,82,84]. Sexual reproduction seems to be rare in ericoid and arbuscular mycorrhizas but common in their free-living relatives [31].</p>

genomes [14,51]. Key loci employed by bacterial parasites to infect host cells and evade host defense often share homologs in mutualist bacteria [10,52,53]. For instance, type III and type IV secretion systems exhibit homologous pathways in both mutualistic and parasitic bacterial lineages [53–55]. Moreover, the bacterium *Aeromonas veronii* uses the same type III secretion systems both to establish beneficial infections within the intestines of leech hosts and to parasitize mammalian hosts [55]. Homology can also be found in loci that protect microbes from host defenses: exo-polysaccharides protect bacterial pathogens against a host’s antimicrobial arsenal, and these molecules seem to offer protection against reactive oxygen species in beneficial plant symbionts as well [56].

Phylogenomic analyses have been employed to investigate genomic differences among whole lineages of bacteria that include both parasites and mutualists. For instance, the Rhizobiales is an order of α -proteobacteria that has diversified from environmental origins into animal and plant pathogens as well as beneficial plant symbionts. Carvalho and colleagues [51] analyzed subsets of loci among these genomes that are shared between harmful and beneficial bacterial lineages versus loci that are unique to one or the other strategy. Whereas few known loci were found to be unique to mutualists or parasites, a larger number of non-housekeeping gene clusters are shared among them, including homologs of infection and virulence loci. However, an important point regarding these loci is that shared ancestry does not necessarily infer like function; the molecular functions of diverged homologs in mutualists and parasites are rarely well understood.

Frequency-dependent selection in beneficial infections

The prediction that mutualistic symbioses will exhibit positive frequency-dependent selection [31,32] has only been tested indirectly and thus far has received mixed empirical support (Box 3). Furthermore, the data for the most part provide information only about selection acting on hosts, not microbes. For example, Parker [33] reviewed data for resistance polymorphism in plants and found that hosts exhibit little polymorphism in loci conferring resistance to beneficial microbes, in contrast to a high level of polymorphism in loci conferring resistance to harmful microbes. These data are consistent with positive frequency-dependent selection on host alleles associated with mutualism and negative frequency-dependent selection on alleles associated with parasitism. By contrast, molecular studies have found evidence of selection for novel alleles (i.e. positive selection) at host loci that modulate beneficial infections in both plants and corals, most probably in response to rapid turnover of genotypes in symbiont populations [41,57].

Another type of data, also considered by Parker [33], is the observation of spatial stability of interactions between hosts and beneficial symbionts (the legume host *Amphicarpa bracteata* and its rhizobial symbionts), in contrast to the rapid turnover of genotypes that can often be observed in host–parasite interactions [58]. Spatial stability of host–microbe pairings is consistent with positive frequency-dependent selection but Parker’s data seem exceptional; other studies in host–microbe systems have failed to find such structure [59].

Evolutionary rate in beneficial microbes

Multiple empirical approaches can examine whether beneficial microbes exhibit evolutionary stasis but each is imperfect. Law and Lewis's use of taxonomic diversity as a proxy for evolutionary rate [31,32] is problematic because of the lack of a universally accepted species concept for microbes, and because microbial speciation rates depend on many factors that are unrelated to interaction with hosts, including regulatory complexity and potential for horizontal gene transfer [36,60,61]. A flood of data since the 1990's has shown that beneficial microbes rarely exhibit the dearth of taxonomic diversity compared to hosts that Law and Lewis found [2,5,8,11,62] (Box 3). At the extreme are microbial lineages known from molecular studies to be hyper-diverse – for instance *Nostoc*, rhizobia and zooxanthellae – that were originally classified as widespread single species based on microscopic analysis [3,63,64]. Yet, at least one notable exception to this pattern exists. Lichens are associations in which diverse fungal partners (mycobionts) form symbioses with depauperate lineages of bacteria and unicellular algae (photobionts). In some cases the photobionts are only represented by a small handful of genotypes [65,66]. Zoller and Lutzoni [66] further showed that some photobionts evolve much more slowly than their mycobiont hosts at homologous loci, consistent with the predictions of Law and Lewis [31]. Reduced evolutionary rate in the photobionts might be favored by the protective conditions of the mycobiont host [31] or by intense infection specificity imposed by hosts [65] but these hypotheses remain to be tested.

To examine the link between host association and molecular evolution more directly, researchers have compared nucleotide substitution rates in housekeeping loci of beneficial microbes versus homologs in free-living relatives. These tests also carry caveats: the evolutionary transition to host association can alter a microbe's effective population size, recombination rate, rate of gene loss [4,50], generation time and thermal and oxidative environments [18], any of which can affect molecular evolutionary rate. A suite of studies has shown that vertically transmitted beneficial bacteria exhibit increased nucleotide substitution rates compared to free-living relatives [4,17–21,52,67,68]. There is debate over the potential drivers of the rapid molecular evolution including the accumulation of mildly deleterious mutations due to small population size within hosts (the nearly neutral theory; [69]), increased overall mutation rate [70] and altered selective pressure within hosts [20], none of which are mutually exclusive. The nearly neutral theory [69] is well supported among these hypotheses because variation in nucleotide substitution rates among vertically transmitted lineages seems consistent with differences in effective population sizes [19,21]. Other predictions of the nearly neutral theory are also supported, such as the evolution of compensatory mutations [21,69]. For instance, heat-shock proteins such as GroEL chaperonins, which help to fold damaged proteins, exhibit strong positive selection in vertically transmitted bacterial lineages, probably as a response to the high mutational load that these bacteria carry [71].

There are only a handful of molecular evolutionary rate studies of environmentally acquired microbial mutualists

that, unlike vertically transmitted microbes, do not exhibit severely reduced population sizes [18]. In dinoflagellate algae that infect marine invertebrates, host-associated lineages exhibit similar [72] or possibly reduced [73] nucleotide substitution rates compared to free-living taxa. Peek and colleagues [18] studied bacterial symbionts of mollusks that include both vertically and environmentally acquired taxa. They also found that the environmentally acquired symbionts exhibited comparatively reduced nucleotide substitution rates compared to free-living taxa [18]. Peek and colleagues [18] evoked the nearly neutral theory [69] to explain this pattern. Their scenario assumes that effective population size and the efficiency of purifying selection are significantly increased in environmentally acquired microbes compared to the free-living taxa [18], which is plausible because environmentally acquired microbes can proliferate both within and outside of their hosts (e.g. [5,11]). An alternate explanation is that hosts that environmentally acquire symbionts evolve specificity of infection (e.g. [74]) that selects against novel symbiont genotypes, similar to the prediction of evolutionary stasis by Law and Lewis.

Overall these data do not provide support for the prediction that beneficial microbes exhibit evolutionary stasis to maintain adaptation to hosts. More recent work on microbial mutualism has shifted focus to consider the mosaic nature of genome evolution in which mutation, natural selection, and evolutionary rate can vary greatly across a genome. One prediction is that microbial loci that directly modulate interactions with host cells should exhibit evolutionary constraint in mutualists compared to parasites [75]. Jiggins and colleagues [75] analyzed nucleotide substitution rates in a gene coding bacterial outer membrane proteins (*wsp*) in mutualistic and parasitic *Wolbachia* (that infect nematodes and arthropods, respectively). They uncovered evidence for positive selection in amino acids expressed in the outer membrane of parasitic species but not mutualists, indicating that arms race dynamics might only occur in parasites [75]. Similarly, Brownlie and colleagues [76] studied both parasitic and mutualistic lineages of *Wolbachia* and found evidence of positive selection in loci that directly interact with host immune function, but such evidence was more prevalent in parasitic than in mutualistic lineages. Dale and Moran [54] analyzed sequence evolution in type III secretion loci: membrane-associated complexes that inject molecules into hosts and can be found in bacterial mutualists and parasites of insects. They found signatures of positive selection in both the mutualistic and parasitic bacterial lineages that they analyzed, consistent with the hypotheses that conflicts of interest can be intense even in beneficial interactions [54]. Finally, Toft and colleagues [77] examined genome-wide substitution rates in two beneficial endosymbiotic bacterial lineages in insects and found that 20–30% of loci exhibited reduced substitution rates compared to free-living bacterial lineages, consistent with selective constraints on these genes. The constrained loci were often related to metabolism and transport of amino acids to the host indicating that the host-symbiont interaction has reduced evolutionary change for pathways that provide benefits to the host [77].

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Two caveats must be kept in mind when interpreting these data. First, *Wolbachia* and related symbionts of insects, from which much of these data come, can rapidly evolve from parasites into mutualists [47], and without detailed phenotypic data this instability could obscure evolutionary signals of mutualism versus parasitism. Second, genome-wide effects of drift in endosymbionts can mask evidence of positive selection acting on specific loci [78], making comparisons between endosymbionts and free-living relatives difficult. Nonetheless, the studies that we review support the view that coadaptation between beneficial microbes and their hosts can constrain the evolutionary rate of some microbial loci, especially for microbial functions that provide crucial services or metabolites to hosts.

Sexual exchange in beneficial microbes

Although some beneficial microbes seem to be typically asexual (such as arbuscular mycorrhizae; [79]), and a few ancient endosymbiotic lineages have lost all recombogenic function [80], overall there is little evidence of a connection between the evolution of asexuality and host association. Diverse beneficial microbes have been discovered to exhibit high rates of genetic interchange, including genome-wide recombination, conjugation of plasmids and genome islands as well as other mechanisms [49,81,82]. Genetic exchange has even been demonstrated in some vertically transmitted endosymbionts [83,84], the microbes that are arguably the most sheltered within their hosts and thus predicted to most benefit from asexuality [31,32]. The only groups for which Law and Lewis found a clear difference in the rate of genetic exchange between mutualists and free-living relatives were the ericoid and arbuscular mycorrhizas [31,32]. However, asexual reproduction is also common in many parasitic and saprotrophic fungi, so its presence in these two groups of mycorrhizal fungi could have more to do with fungal biology than with their mutualistic lifestyles [85]. Nonetheless, these data are mostly qualitative and more rigorous tests are needed (see below).

Conclusions

Microbial mutualists are more similar to parasites in their evolutionary patterns than previous paradigms have suggested [31,32]. Firstly, genomic data indicate that basic mechanisms of infection, proliferation and defense within hosts can overlap extensively between parasitic and mutualistic microbes [10,15,51,53]. Evidence of rapid symbiont turnover has been found in several types of beneficial infections [36,41,86] indicating the potential that some mutualistic symbioses, can result in negative frequency-dependent selection (similar to host-parasite interactions). Moreover, in contrast to predictions of evolutionary stasis [31,32], some of the most intimate microbial mutualisms – vertically transmitted endosymbioses – commonly exhibit rapid molecular evolution [4,17–21,67,68]. In these cases, drift caused by reduced effective population size is likely to be a more important driver than host adaptation. Finally, mechanisms and evidence of sexual exchange have been found across a wide variety of mutualistic microbes [49,81–84].

Despite their limitations, the ‘Mutualistic environment’ models [31–33] have helped to identify some important differences between beneficial and harmful infections. Some evidence does exist for positive frequency-dependent selection in mutualistic associations [33], in contrast to abundant evidence for negative frequency-dependent selection in parasitisms [26–28]. In addition, some microbial loci involved in mutualistic associations exhibit suppressed evolutionary rates [75,77], in contrast to heightened evolutionary rates in many loci involved in host-parasite interactions [29].

New paradigms for the evolution of beneficial infections must take into account several evolutionarily significant aspects of microbes that were not previously emphasized. First, predictions regarding evolutionary rate must allow for both the effects of population size (acting through genetic drift) and the mosaic nature of genome evolution. Predictions should also take into account the diversity of

Box 4. Outstanding questions

- **To what extent do microbe loci that modulate host interaction functions exhibit increased evolutionary constraint in mutualists compared to pathogens (e.g. [75,77])?** This question is related to the hypothesis that microbial mutualists exhibit evolutionary stasis because of beneficial coadaptation with hosts [31]. Arms race evolution is evident from rapid evolution and positive selection in key host-association loci of microbial pathogens [29,75,76], and one research approach could examine whether homologous loci experience similar or opposing selection pressures in beneficial microbes. Environmentally acquired microbial mutualists have received very little empirical attention compared to vertically transmitted taxa, and would be particularly interesting subjects for these comparisons because conflicts of interest with hosts are predicted to be more intense where the fitness interests of hosts and microbes become unlinked with each new round of transmission [37,43]. Genomic approaches offer the potential to survey loci involved in host-related functions, such as infection, proliferation, and transmission, to identify those experiencing evolutionary constraint versus positive selection [51,76,77]. These loci could then become the subjects of more focused analyses comparing their evolution in mutualists and related parasites and free-living taxa.
- **Do molecular mechanisms of genetic exchange exhibit an evolutionary reduction in mutualistic compared to related free-living or harmful microbes?** Microbial mutualists are not mostly asexual, as Law and Lewis originally suggested, but the evolution of sex in microbes (e.g. recombination rate, conjugation, horizontal gene transfer) and its relationship to evolutionary transitions in host association remains poorly understood. In particular, it is unknown whether adaptation to hosts in mutualists is modulated by an evolutionary reduction in the rate of genetic exchange. Empirical tests, carried out in a phylogenetic framework, can compare quantitative measures of recombination rate or horizontal gene transfer among related microbes that vary in host association status.
- **What are the key genomic differences between related lineages of microbial mutualists, parasites and free-living taxa in terms of genome content and gene function?** Initial surveys that have focused on bacterial genomes have struggled to identify key loci that drive virulence in microbial parasites and that do not share homologs in non-parasitic lineages [14,51]. Future research should examine changes in the intensity of selection within key loci involved in host-symbiont interactions during transitions between free-living, parasitic, and mutualistic lifestyles (such as type III secretion systems and outer-membrane proteins). Moreover, studies should focus on functional divergence between homologs in mutualists and parasites.

mutualistic interactions (Box 1), and attempt to identify why different host–mutualist associations exhibit different patterns (e.g. the low species diversity of photobionts within lichen [65,66] versus high diversity of rhizobia within legumes [5]). For instance, aspects of mutualisms that modulate the degree to which the partners' fitness interests are linked, such as the mode of transmission and the frequency of multiple infections, should also influence the extent to which these mutualisms differ from parasitisms in their evolutionary patterns. Given the spatial heterogeneity of communities, accurate predictions might also depend on understanding how the presence of other species (e.g. parasitoids [45] or other symbionts [87]) might shift a host–symbiont interaction along the parasitism–mutualism continuum: interactions that shift frequently should be expected to exhibit little divergence from parasitisms in their evolutionary signatures. Finally, future paradigms must take into account conflicts of interests between mutualistic symbionts and their hosts that can occur even in strictly vertically-transmitted mutualisms. The development of these new paradigms will require careful research focused on answering outstanding questions about the evolutionary and genomic differences between beneficial and harmful symbionts (Box 4).

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