Microbial evolution and transitions along the parasite–mutualist continuum

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Abstract | Virtually all plants and animals, including humans, are home to symbiotic microorganisms. Symbiotic interactions can be neutral, harmful or have beneficial effects on the host organism. However, growing evidence suggests that microbial symbionts can evolve rapidly, resulting in drastic transitions along the parasite–mutualist continuum. In this Review, we integrate theoretical and empirical findings to discuss the mechanisms underpinning these evolutionary shifts, as well as the ecological drivers and why some host–microorganism interactions may be stuck at the end of the continuum. In addition to having biomedical consequences, understanding the dynamic life of microorganisms reveals how symbioses can shape an organism’s biology and the entire community, particularly in a changing world.

Parasitic and mutualistic microbial symbioses exist widely in nature. These interactions occur when microorganisms (that is, bacteria, fungi and viruses) take up residence in or on animals or plants, and cause damage or confer benefits to the host. Parasitic microorganisms (including pathogens) can exploit the host, and in doing so, cause harm. The term mutualist classically refers to any organism in a mutually beneficial relationship with another. However, the assumed benefits are rarely empirically tested for the symbiont. There is thus an emerging awareness that many putative mutualisms may even be hosts exploiting symbionts, in an interaction referred to as inverted parasitism.

The designation of entities as ‘parasite’ or ‘mutualist’ implies a simple binary system where species incur positive or negative impacts on fitness during interactions. However, these terms represent ends of a continuum along which an interaction between a host and symbiont can shift. These transitions occur as the relative benefits and costs to each species in the relationship strengthen or weaken (Fig. 1) across ecological or evolutionary time. Transitions can be driven by changes in the environment and ecology of the interacting species or communities. At the centre of the continuum sit commensals, which benefit from the interaction with hosts, but do not cause a detectable cost.

The concept of the parasite–mutualist continuum dates back several decades. An early discussion by Ewald focused on the fundamental role of transmission route in driving evolutionary transitions between parasitism and mutualism in symbiotic associations. The conditionality of symbiotic interactions was later highlighted by Bronstein. She reviewed evidence that the costs and benefits of interspecific interactions vary greatly with ecological context, and thus the outcome of a symbiosis can change throughout an organism’s lifetime.

Evolution of microorganisms into parasites or mutualists. Microorganisms can rapidly adapt to new environments. Short generation times, large population sizes and high mutation rates combined with genome flexibility all facilitate accelerated microbial evolution. Furthermore, their capacity for plastic responses and the dynamic nature of the communities that microorganisms are nested and interact within provide further routes for changing costs and benefits of association with hosts.

Free-living environmental microorganisms, which do not associate with hosts, were the progenitors for all symbiont diversity observed today. Free-living microorganisms can evolve to be parasites or mutualists. A new host-associated lifestyle often remains facultative for the microorganism, but in some cases the microorganism evolves an obligate dependency on the host. Transitions from free-living to host association are sometimes facilitated by horizontal transfer of genes, often encoding traits that facilitate immediate exploitation of, or benefit to, hosts (for example, immune evasion, toxin production, nitrogen fixation and bioluminescence). Once associated with a host, symbiotic interactions can shift along the continuum. For instance, parasites can evolve to be less antagonistic to hosts. Reduced antagonism is thought to be favoured if alternative hosts are rarely available or if transmission of the parasite is enhanced by increases in host fitness. Molecular phylogenetics corroborates this trajectory, showing that parasites have frequently served as progenitors for the independent descent of symbionts that now exhibit mutualistic traits. In this context, microorganisms
A microbial lifestyle not dependent on association with a host for long-term survival and replication; this is the ancestral state of all symbionts.

Mobile genetic elements (MGEs): Sequences of genetic material that can be exchanged between chromosomes or organisms via either their own mobilizing machinery or that of their host. Examples include transposable elements, plasmids and phages.

Horizontal gene transfer (HGT): The movement of genetic material between organisms that does not flow from parent to offspring.

extreme ends of the continuum. Moreover, we focus the Review on eukaryotic host–microorganism symbioses; however, we note that microbial interactions with mobile genetic elements (MGEs) can be analogous to symbionts (BOX 2) given the ability of these elements to confer beneficial traits and cause harm to bacterial hosts.

**Mechanisms of evolution along the continuum**

The gradual emergence of microbial mutualists from parasitic ancestors and the rapid leaps in symbiont phenotypes observed in real time provide fascinating insights into the proliferation of microbial symbiotic diversity. The genetic changes involved in microbial evolution are key contributors to the formation of mutualisms and parasitisms and their transitions along the symbiotic continuum. Mechanisms that result in these changes include, for example, selection on existing genetic variation, de novo mutations and genome rearrangements. These events often involve MGEs — such as plasmids, transposons, insertion elements and phages — coding for traits that are beneficial or harmful to hosts during their interaction.

Fluctuations between microbial parasitism and mutualism can involve selection on existing variation. Through experimental evolution of the bacterial symbiont *Parachlamydia acanthamoebae* and its protist host *Acanthamoeba* sp., one study observed an evolutionary shift of the microbial symbiont towards parasitism under horizontal transmission conditions. The molecular basis of this transition was a pronounced increase in the frequency of specific genetic variants within the original symbiont population, alongside marked changes in the expression of machinery necessary for manipulating host cells, such as the type III secretion system (T3SS).

Selection on de novo mutations in bacterial populations has also been detected in evolution experiments, resulting in movement along the continuum. In these cases, experiments are started by propagating a single clone in hosts. In one study, a clonal population of *Enterococcus faecalis* was introduced into nematode host populations, and mutations that arose favoured enhanced production of reactive oxygen species. This phenotype allowed *E. faecalis* to become highly beneficial to hosts, as production of these antimicrobials suppressed infection by *Staphylococcus aureus*. A similar direction of travel, but from parasite to commensal, has been observed in nematode host populations by evolving *Pseudomonas aeruginosa* from a single clone. Conversely, within the guts of old mice, mutations arising in clones of commensal *Escherichia coli* may have resulted in evolution towards pathogenicity. In comparison with evolution within young mice, mutational targets linked to stress-related functions and associated with virulence were under strong selection in the inflamed guts of older mice. Mutation might have a
prominent role in transitions when symbionts have a low initial diversity upon colonization. This situation could occur naturally when symbionts have a low infectious dose or when transmission causes population bottlenecks (see section on Transmission below).

Wide-ranging genetic changes — HGT, gene loss and genome rearrangements — have had a profound role in *Yersinia pestis* becoming more virulent and adapting to new host species \(^{15,20,21}\). *Y. pestis* is the causative agent of plague in mammalian and arthropod hosts. It is thought to have diverged from its less harmful ancestor *Yersinia pseudotuberculosis* 1,500–55,000 years ago \(^{2,63}\). Sequencing of isolates of the two species revealed that both HGT and insertion sequence-mediated genome rearrangements and deletions facilitated *Y. pestis* evolution \(^{15,20,21}\). The bacterium acquired two plasmids, namely pMT1 and pPCP1, making it more virulent compared with its *Y. pseudotuberculosis* ancestor. The former plasmid carries the *ymt* gene encoding *Yersinia* murine toxin, required for the colonization of the flea host \(^{4,25}\), and the capsular antigen fraction 1, which inhibits phagocytosis \(^{20}\). These acquisitions contributed to the evolution of *Y. pestis* towards greater virulence. Adaptation of the parasite to new hosts was mediated by genome rearrangements, particularly via insertion sequences and gene loss. Gene loss was crucial in reducing the toxicity of *Y. pestis* to the flea vector, allowing biofilm to develop in the flea foregut \(^{20}\). Gene disruption by insertion sequences, in combination with deletion events, point mutations and frameshifts, further created an extensive number of pseudogenes within the *Y. pestis* genome \(^{20,201}\). Altogether, these genetic changes facilitated a shift in lifestyle, from a less harmful mammalian enteropathogen to systemic pathogen of both mammalian and arthropod hosts.

Infection by various phages (mostly lytic, λ-like phages) along with other MGEs facilitated the divergence of the highly pathogenic enterohaemorrhagic *E. coli* strain O157 Sakai from its ancestor. The commensal *E. coli* strain K12 is also descended from this common ancestor \(^{15}\). In strain O157 Sakai, prophages and prophage-like elements encode a variety of virulence-related genes — adhesins, tellurite resistance genes and urease — contributing to the acquisition of virulence factors that have determined this bacterium’s trajectory towards increased virulence in humans. One of these elements also encodes the major virulence factor, the locus of enterocyte effacement (LEE), which is responsible for bacterial attachment followed by development of the disease-causing effacing lesions in the intestine \(^{26}\). Lambda-like phages on the Sakai chromosome also encode the destructive Shiga toxin, as well as proteins involved in serum resistance and cell adhesion. Having become integral to the organism’s virulence in this way, the prophages themselves have transitioned from parasitic to mutualistic elements within the O157 Sakai genome (for further discussion of MGEs as symbionts, see BOX 2).

How commonly do shifts across the continuum occur owing to de novo mutation or machinery acquired by HGT? Host environments with complex, often open, microbial communities, such as the mammalian gut, might generate more extensive opportunities for HGT \(^{7,22}\). For example, phage-driven HGT from the resident community can dictate the evolution of invading strains \(^{73}\) and instigate change more rapidly than is achievable by mutation accumulation \(^{1}\). HGT has had a considerable role in major evolutionary transitions of living organisms; it is increasingly confirmed as a dominant force in the evolution of host–symbiont associations \(^{23,24,25,26,27}\). Yet, for symbions nested within simple microbial communities (for example, intracellular environments), scarce opportunities for HGT may mean de novo mutation is more likely to underpin shifts along the continuum. Studies reporting selection on de novo mutation during transition \(^{28,29,30}\) highlight the power of this genetic means to generate remarkable change on the continuum. These experiments typically involve a small number of microbial species and/or low levels of initial genetic diversity upon colonization. When incorporating a host background

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**Box 1 | Two approaches to evaluating evolution along the parasite–mutualist continuum**

**Phylogenetic inference**

There are challenges to judging transitions in symbiosis because ancestral partnerships no longer exist for direct comparison. Interactions that now appear mutualistic may actually reflect the result of a long period of conflict resolution or the evolution of tolerance by the host. Phylogenetic inference can shed light on the evolutionary history of transitions on the parasite–mutualist continuum. Techniques such as ancestral state reconstruction and its extensions infer characteristics of ancestral taxa based on traits exhibited by extant descendants \(^{15,20,21}\). In this way, symbiotic phenotypes of ancestors (for example, parasite, mutualist, commensal or free-living) can be recovered and used to infer the origins and breakdowns of associations on the continuum, in addition to the rate of such transitions \(^{25}\). Such approaches are heavily contingent on the quality of the underlying phylogenetic tree, and reconstruction accuracy declines with increasing evolutionary time \(^{26}\). However, for many lineages of bacterial symbionts this approach has been used powerfully to demonstrate the marked rarity of reversions from mutualism to parasitism over evolutionary timescales \(^{27,28}\).

**Experimental evolution**

Experimental evolution permits the direct testing of hypotheses related to the tempo and pattern of the evolution of species interactions. This approach allows for evolution to be observed in real time. An added advantage in some systems is an ability to cryopreserve the eukaryotic host (for example, *Caenorhabditis elegans* \(^{29,30}\) and *Paramecium bursaria* \(^{31}\)) and associated microbial lineages for subsequent analysis. This characteristic allows the fitness benefit or harm for both species to be compared with past and future archived generations, for example, via time shift assays \(^{27,28}\).

In an evolution experiment, the source of selection can be hypothesized and manipulated. For example, this approach could be used to determine whether the presence or absence of an enemy could affect the position of a defensive symbiosis along the continuum \(^{32}\), as well as whether the evolution of the eukaryotic host or the microbial symbiont, or their coevolution was responsible for the shift \(^{33}\). Subsets of the population can be used to establish the next generation. One focal species can be evolved and others kept in evolutionary stasis by adding from an ancestral population each generation. Alternatively, additional community members can be reciprocally evolved, opening the arena for coevolutionary dynamics between two or more species \(^{34}\). The process continues for generations. At the end, phenotypic and genomic comparisons can be made between ancestral and evolved populations, and also across replicates, to assess convergence or divergence in transition outcome and the genetic basis.

Candidate molecular targets in evolved lineages can be identified for manipulation and further experimentation. Moreover, follow-on genomic analysis can be powerfully combined with phenotypic assays across evolutionary time to identify the mechanism of relative benefit or cost for each species, as well as to confirm phenotypic traits under selection. One caveat is that experimental evolution might be less likely to yield increases in parasite virulence given the potential for breaking apart of the virulence–transmission trade-off at passage points \(^{35}\).
Mobile genetic elements (MGEs) can cause genomic change in their microbial hosts. These changes can affect the position of the microorganism–eukaryotic host relationship on the parasite–mutualist continuum by coding for traits that harm or benefit microbial hosts. On a smaller scale, MGEs are analogous to symbionts27,28 as they are entities with their own evolutionary interests that can parasitize hosts or confer beneficial traits that promote innovation. The effects they have on microbial host fitness can change.

Many nosocomial pathogens have acquired antibiotic resistance genes through horizontal gene transfer29, gaining a survival advantage in the presence of certain antibiotics. In the absence of the corresponding antibiotic, however, a resistance-conferring MGE can become costly to its host. For example, when large low-copy-number plasmids are cumbersome to their host, these plasmids force their maintenance through the action of resolution systems, partitioning systems and post-segregational killing systems. The latter of these includes toxin–antitoxin systems, encoding both a stable protein toxin and a less stable, but more abundant antitoxin. If a plasmid fails to be inherited by a daughter cell, the antitoxin will rapidly degrade in the host, leaving it susceptible to being killed by the toxin29. The transition of MGEs from beneficial elements conferring a survival advantage to parasites can take place over very short evolutionary timescales. In turn, in the face of antibiotic treatment and other clinical interventions, MGEs can drive the evolution of their bacterial hosts towards higher virulence over an equally short period of time30,31.

MGEs are not always maintained through natural selection. The genome of Wolbachia pipientis wMel, an obligate intracellular symbiont of the fruitfly Drosophila melanogaster, is highly streamlined from extensive gene loss during adaptation to its host; however, it is also overrun with MGEs32. Repeated population bottlenecks resulting in genetic drift and inefficient natural selection33 likely contribute to the extensive maintenance of MGEs in this genome and those of other heritable symbionts34. These elements may have contributed to the substantial phenotypic diversity among Wolbachia strains, fundamentally shaping Wolbachia evolution35. In this instance, MGEs are parasitic elements maintained within the population effectively by accident via transmission of Wolbachia from one host to the next. Ultimately, it is unclear whether these elements will cross the parasite–mutualist continuum and become permanent components of Wolbachia genomes.

For some microbial hosts, the acquisition of deleterious MGEs can be partially rescued via compensatory evolution, leading to a type of host tolerance. In such cases, the association is maintained but the host ameliorates the cost, as shown for Pseudomonas fluorescens and a mega-plasmid conferring mercury resistance. In low-mercury environments, the plasmid is costly, yet experimental evolution across a mercury gradient showed P. fluorescens consistently compensated via mutation in the gacA–gacS two-component system, downregulating chromosomal and plasmid gene expression and relieving translational cost36. Such compensatory evolution may also explain the persistence of context-dependent mutualisms in environments where they do not benefit hosts. MGEs can also become ‘immortalized’ in host lineages. Once genomic parasites, they can become indispensable components of the host genome that are ultimately passed on to daughter cells. Vestigial MGEs in the form of cryptic phages, ancient regions of viral DNA and disrupted transposon sequences or pseudogenes can be found immortalized in the genomes of organisms throughout the tree of life37. Bacterial chromosomes, for example, can contain as much as 20% phage DNA38,39. Once parasites to their hosts, these MGEs have infected the genomes of host organisms, maintained their stability as they coevolve with their host (forcibly in some cases, for example, toxin–antitoxin systems) and finally been irreversibly integrated into the genome. Integration can occur by accident during genome rearrangements, recombination, population bottlenecks and speciation events35, or by natural selection because of a fitness benefit on which the host has become dependent40. The ubiquitous presence of vestigial viral DNA in the cells of all organisms41–43 is a prime example, demonstrating how MGEs have been formative in the evolution of organisms, just like many eukaryotic host–microbial symbioses. MGEs leave behind remnants of DNA in host genomes like partial segments of an ancient diary.

MGEs therefore possess the capability themselves to go from genomic parasites to mutualistic or commensal components of the genome. In many situations, this process can also drive the evolution of their bacterial hosts along the continuum. MGEs have forcibly maintained their interaction with bacteria in some cases, while in others, their maintenance has been a by-product of environmental conditions or population bottlenecks. They represent fascinating examples of entities that can be both effectors and subjects of evolutionary transitions along the parasite–mutualist continuum.

Drivers of evolution along the continuum

Ecological sources of selection can drive microbial symbiont evolution towards increasing host benefits (TABLE 1) or harm (TABLE 2). Shifts occur across generations as microbial symbionts adapt to life in a new host species, encounter different transmission opportunities and face hosts that reciprocally evolve in response. The presence or absence of additional interacting species in the community can also drive evolutionary change in a host–symbiont relationship owing to changing distribution of net benefits and costs across the community. Essentially, given a strong source of selection, genetic change can occur within just a handful of microbial generations. These transitions are often investigated using experimental evolution or over macro-evolutionary timescales via phylogenetic comparisons (BOX 1).

Novel hosts. Microorganisms frequently encounter novel host environments. They can jump across species boundaries or colonize hosts from pools of free-living environmental microorganisms. Novel infections can generate new diversity on the symbiosis continuum through divergence and speciation41. High-profile cases of host shifts, such as the recent SARS-CoV-2 pandemic42, highlight the potential for investigating evolutionary changes in virulence upon emergence43–45. New associations are often maladaptive for both host and parasite44, and associations can move unpredictably on the continuum or burn out. This trajectory has been observed in emergencies of avian influenza virus, where case fatality rates can be high but human-to-human transmission is low46.

Shifts between host species, possibly driven by HGT of virulence-associated genes, appear to have been important in the emergence of the Q fever parasite, Coxiella burnetii47,48. This proposed mutualist–to-parasite transition is a complex case for which the full evolutionary story remains unknown. However, phylogenetic analysis suggests that this highly infectious bacterium recently emerged from a clade of vertically transmitted mutualistic endosymbionts of ticks47. C. burnetii may have evolved mechanisms to infect vertebrate cells, persist in the environment and be airborne-transmitted. These
| Transition | Host | Symbiont | Association | Condition | Mechanism and evidence | Approach | Refs |
|------------|------|----------|-------------|-----------|------------------------|----------|------|
| P → P (−) | Ciliate (Paramecium caudatum) | Holospora undulata | Mixed mode transmitted parasite | Low host density | Lower virulence and increased VT frequency | Experimental | 210 |
| P → P (−) | Actinomyces odontolyticus | Nanosynbacter lyticus (TM7x) | Epibont parasite | Naive host and co-culture passage | Host susceptibility rapidly reduced | Experimental | 151 |
| P → P (−) | Escherichia coli | F1 phage | Parasitic phage | VT only | Less virulent variants favoured | Experimental | 217 |
| P → P (−) | European rabbit (Oryctolagus cuniculus) | Myxoma virus | Parasite | Novel host | Increased interferon antiviral activity (host); greater transmission traded off with virulence (virus) | Field sampling | 84, 25 |
| P → P (−) | Nematode (C. elegans) | Staphylococcus aureus | Parasite | Pathogen coevolved with defensive microorganism | Siderophore production reduced | Experimental | 144 |
| P → P (−) | Mouse (Mus musculus) | Friend virus | Parasite | Heterogeneity in host resistance | Resistant hosts drove parasite specialization, reduced mean virulence across host population | Experimental | 219 |
| P → P (−) | Diamond-back moth (Plutella xylostella) | Enterobacter cloacae | Gut symbiont | Pathogen exposure | Reduced virulence in some lineages | Experimental | 220 |
| P → P (−) | Barley (Hordeum vulgare) | Barley stripe mosaic virus | Plant parasite | VT only | Substantial reduction in virulence | Experimental | 111 |
| P → C | Nematode (C. elegans) | Pseudomonas aeruginosa | Gut parasite | Serial passage | Mutation in global regulator lasR and polymerase gene rpoB | Experimental | 49 |
| P → C | Legume (Mimosa pudica) |Ralstonia solanacearum and rhizobial plasmid | Root nodulation | HGT and selection from emergent nodules | T3SS (hrcV) and master virulence regulator (hrpG) inactivated | Experimental | 221 |
| P → M | Squid (Euprymna scolopes) | Vibrio fischeri | Bioluminescence | NA | Inferred evolution from parasitic ancestors | Phylogenetic | 15, 22 |
| P → M | Nematode (C. elegans) | Enterococcus faecalis | Defensive microorganism | Pathogen exposure | Increased antimicrobial superoxide production | Experimental | 40 |
| P → M | Mouse (M. musculus) | Candida albicans | Gut symbiont | Gut microbiota absent | Filamentation loss, increased cytokine response, host protection against infection | Experimental | 45 |
| P → M | Fruitfly (Drosophila simulans) | Wolbachia (wRI) | Reproductive parasite | VT and reproductive manipulation | Fecundity benefit over uninfected hosts | Experimental, field sampling | 121 |
| P → M | Cicadas (Cicadoidea spp.) | Ophiocordyceps fungi | Nutrient provisioning | Genomic decay of existing symbiont | Evolution from pathogens inferred; took over amino acid synthesis | Phylogenetic, field sampling | 105 |
| P → M | Pea aphid (Acyrthosiphon pisum) | Hamiltonella defensa | Defensive microorganism | NA | Putative parasite loci remain (T3SS and toxin homologues) | Comparative genomic, phylogenetic | 121 |
traits are unlikely to be found in the arthropod-restricted ancestors. Ticks feeding on vertebrates likely provided the ecological bridge. Similar transitions occurred within Sodalis-allied symbionts, a group of host-restricted bacteria common to insects including the tsetse fly vector. A free-living Sodalis sp. was isolated after a person suffered a wound from a tree branch, and this serendipitous finding provided evidence that symbiont lineages emerged from environmental ancestors. Early vectoring of these environmental strains by insects was likely pivotal in the evolution of the beneficial, heritable Sodalis endosymbionts observed today.

Novel species interactions can drive rapid innovation. This might particularly be the case if a microorganism bears characteristics that can provide instant benefits. Microorganisms encoding functions of light generation, photosynthesis, nitrogen fixation or antimicrobials may provide such rapid benefits. These characteristics may be...
remodelled (or act as pre-adaptations) for transitions in symbioses. Such repurposing may have occurred in the antifungal-producing *Burkholderia* symbionts associated with Lagriinae beetles. *Burkholderia* symbionts appear to have transitioned from a plant parasite to insect mutualist. In this context, secondary metabolites previously used as virulence factors against plants may have been repurposed for antifungal defence on beetle eggs. Additional

Table 2 | Studies reporting evolution of symbioses towards the parasitism end of the continuum

| Transition | Host | Symbiont | Association | Condition | Mechanism and evidence | Approach | Refs |
|------------|------|----------|-------------|-----------|------------------------|----------|------|
| M → M (−) | Legume (Ensi fer medicae) | Rhizobia | Nitrogen-fixing | Host choice blocked | Cheater strains favoured | Experimental | 29 |
| M → M (−) | Legume (Trifolium spp.) | Rhizobia | Nitrogen-fixing | Elevated nitrogen | Reduced cooperation under high nitrogen | Experimental | 29 |
| M → P | Vertebrate spp. | *Coxiella burnetii* | Intracellular parasite | Host shift | HGT of virulence-associated genes suggested | Phylogenetic | 30 |
| M → P | Jelly fish (Cassiopea xamachana) | Alga (Symbiodinium microadriaticum) | Photosynthetic per New | HT only | Greater proliferation in MT dispersal rates | Experimental | 10 |
| M → P | Plant spp. | *Agrobacterium* spp. | Plant parasite | NA | HGT of virulence loci | Phylogenetic | 29,31 |
| M → P | Plant spp. | *Pseudomonas syringae* | Plant parasite | NA | HGT of hopZ T3SS effectors | Phylogenetic | 25,79 |
| M → P | *Escherichia coli* | M13 phage | Growth benefit | Host background | Parasitic when shifted to host ancestor | Experimental | 28 |
| M → P | *E. coli* | F1 phage | Parasitic phase | HT allowed | Antagonistic variants favour | Experimental | 27 |
| C → P | Pill bug (Armadillidium vulgare) | *Wolbachia* (wVulC) | VT endosymbiont | HT only | Titre increased in non-germline-associated tissue | Experimental | 110 |
| C → P | In vitro immune environment | *E. coli* | Commensal strain | Macrophage pressure | Heightened macrophage evasion and delayed phagosome maturation, via TE insertion | Experimental | 232 |
| C → P | *Arabidopsis thaliana* | *Pseudomonas fluorescens* species complex | Rhizosphere associated | NA | Gain of putative pathogenicity island | Comparative genomics, phylogenetic | 99 |
| C → P | Plant spp. | *Rhodococcus* spp. | Plant associated | NA | Gain of virulence plasmid (pFID188), host growth inhibition | Experimental, comparative genomics, phylogenetic | 59 |
| P → P (+) | Plant spp. | Xanthomonadaceae spp. | Phytopathogen | NA | Gain of hydrolase gene (cbsA); localized parasite become systemic | Comparative genomics, phylogenetic | 54 |
| P → P (+) | Barley (Hordeum vulgare) | Barley stripe mosaic virus | Plant parasite | HT only | Increased virulence, independent of titre | Experimental | 11 |
| P → P (+) | House finch (Haemorhous mexicanus) | *Mycoplasma gallisepticum* | Emerging parasite | Adaptation to novel host | Linear increase in virulence since shift | Natural sampling | 83 |
| P → P (+) | Mouse (Mus musculus) | *Cryptococcus neoformans* | Opportunistic parasite | Serial passage | Increased expression of iron reductase and host mortality | Experimental | 233 |
| P → P (+) | Amoebae (Acanthamoeba sp.) | *Parachlamydia acanthamoebae* | Obligate intracellular symbiont | HT only | Enhanced infectivity and virulence, T3SS upregulated | Experimental | 46 |
| P → P (+) | Mammal spp. | *Yersinia pestis* | Enteric parasite | NA | HGT of plasmids (pMT1 and pPCP1), increased transmissibility by fleas and virulence to mammals | Genomic | 85 |

(−), reduced; (+), elevated (for example, P → P (+) indicates transition towards increased parasitism); C, commensalism; HGT, horizontal gene transfer; HT, horizontal transmission; M, mutualism; NA, specific drivers of transition unaccounted for owing to timescale; P, parasitism; T3SS, type III secretion system; TE, transposable element; VT, vertical transmission. Transitions involve increased virulence or reduced benefit of the symbiotic relationship to hosts over time. General evidence to support the inferred transition, including the molecular mechanism if known.
Evidence comes from marine hosts, including within the bulbs of anglerfish and the Vibrio fischeri-filled light organs of bobtail squid. These hosts benefit from these bioluminescent bacteria to lure prey and avoid predation, respectively, and the symbionts often retain the capacity to live freely, or persist in the environment. Transmission opportunities. Transmission mode has been considered to predict the direction of a symbiont’s evolution on the continuum. When horizontally transmitted symbionts can move between unrelated host individuals, the fitness interests between species are uncoupled, a scenario thought to favour parasitism. The degree of harm caused to hosts from infection is often framed by the virulence–transmission trade-off. The relationship assumes that virulence — the reduction in host fitness caused by parasite infection — is costly to the parasite as host resources are needed for replication. The cost of harming the host too much or too soon from replication might result in less transmission. Thus, it is predicted that transmission should be highest at intermediate virulence, which balances the costs of within-host replication and infectious period length. This model is particularly pertinent for symbionts that rely on a mobile host for transmission (for example, socially transmitted microorganisms). Those that do not (for example, vector- and water-borne microorganisms) can bypass trade-offs between virulence and transmission. This conventional model goes some way to hypothesizing on general patterns of virulence, yet several extensions and alternatives have been suggested.

It has been suggested that mutualists may evolve from parasitic ancestors when the frequency of horizontal transmission routes is reduced or lost. If vertical transmission is the remaining dominant mode of transmission then the fitness of host and symbiont can become tightly coupled, reducing the arena for evolutionary conflict and thereby favouring selection for mutual benefit. Mutualisms involving symbiont inheritance are predicted to be stable on the continuum and unlikely to revert to parasitism. But exclusively vertical transmission can endanger associations via genetic bottlenecks (see section on Stuck at the end of the line). Clearly, becoming inherited is not the sole route by which bacterial mutualists evolve. Comparative analysis has found no evidence for vertical transmission preceding the origin of mutualism. Many mutualisms involve horizontal transmission such as conjugative plasmids in bacterial populations and the vast networks of mycorrhizae that improve plant productivity. In particular, evolution of defensive traits in symbionts are proposed to be facilitated by the genetic diversity and selection for innovation promoted by horizontal transmission. Many horizontally transmitted microbial symbionts are obligate for host fitness, but many can be facultative and confer costs in different environments.

Conversely, not all inherited microorganisms become mutualists. Wolbachia, Spiroplasma and Arsenophonus species are common inherited parasites that manipulate host reproduction, maximizing resource allocation to the transmitting host sex (females) by feminizing hosts or killing their sons. However, theory suggests that the spread of such reproductive parasites will be enhanced by the evolution of traits that benefit hosts. A beneficial trait (that is, defence) may even interact with a parasitic trait (that is, reproductive manipulation) to completely exclude a natural enemy. Indeed, cryptic benefits are now found in several systems, and there is evidence that some reproductive parasites may need to also transmit horizontally just to persist.

Transmission as a determinant of the location of a symbiosis along the continuum is complex. There are numerous exceptions to classical theory. Nonetheless, experimental manipulation of transmission modes finds general support for the theory that horizontal transmission can select for parasitism and vertical transmission for reduced antagonism. In a symbiosis between a jellyfish and the alga Symbiodinium microadriaticum, cooperative traits, including growth enhancement, were selected when transmission was restricted to heritable routes. Such cooperative traits are fundamental for stable mutualisms, protecting against transitions to parasitism or abandonment events. In the reverse experiment, restriction of the alga to horizontal transmission selected for faster proliferation and dispersal (traits associated with parasitism), and declines in host fitness were detected. Such findings are mirrored across terrestrial systems. The common pill bug hosts a Wolbachia strain (wVulC) that feminizes genetic males. Blocking the typical vertical route, and mimicking horizontal transmission, saw systemic increases in Wolbachia (wVulC) density and a drastic transition from a benign partner to a highly virulent one.

The community. The drivers of transitions along the parasite–mutualist continuum can be complex and stem from the ecological and evolutionary movements of many different players. Defensive symbiosis, whereby there are at least three interacting species (host, defensive symbiont and an attacking enemy) is particularly dynamic along the continuum in response to community composition changes. The absence of the symbiont or enemy can have evolutionary consequences for other species in the community, even without direct interactions. Co-infections in hosts can also influence transitions in the symbiosis by providing new phenotypes via HGT of genetic material (for example, symbiosis islands, plasmids and phages).

The impact of community complexity is demonstrated by the bacterium Hamiltonella defensa and its lysogenic phage, APSE. This association protects host aphids against parasitoid wasps (FIG. 2). In this context, the fitness benefit afforded to the aphid host is contingent on parasitoid presence — in its absence, H. defensa with APSE phage is costly to the aphid. The mechanism of protection (toxin production) hinges on the initial lateral transfer of phage from a co-infecting symbiont. Subsequent loss of the phage can move the interaction between H. defensa and aphids back towards parasitism. Experimental evolution and field studies have captured how microorganisms, even parasitic ones, can evolve rapidly to protect their hosts when collectively threatened, often
crossing the parasite–mutualist continuum in the process. In Caenorhabditis elegans nematodes, a mildly parasitic gut bacterium was shown to evolve an enhanced ability to protect against infection by a more virulent parasite\(^a\). In the parasite’s absence, the gut bacterium did not emerge as a microbial line of host defence.

Additional symbions, with previously unknown effects, are increasingly being identified even in iconic ‘two-player’ symbioses, such as corals\(^b\) and lichens\(^c,d\). It is thus not surprising that the complexity of a host’s whole microbiota (which often includes a diverse repertoire of bacteria, fungi and viruses) can interact to produce new outcomes for individual strains, species and the community as a whole. Members of the microbiota compete and cooperate in a myriad of ways\(^e\), influencing the virulence of one another via processes such as the suppression of public goods\(^f\), or the facilitation of biofilm formation\(^g\) and epithelial translocation\(^h\). The passage of Candida albicans in mice lacking gut microbiota has highlighted the role of communities in determining fate on the parasite–mutualist continuum. In the absence of a gut microbiota, C. albicans mutants emerge that are defective in hyphal formation, no longer requiring it for competition against other microbiota members. When compared with the wild-type ancestor that coexists with a microbiota, these C. albicans mutants are less virulent and protect their hosts against Aspergillus fumigatus infection in a manner independent of host adaptive immunity\(^i\). This transition from pathobiont to conditional mutualist in this context appears to hinge on the absence of competing microorganisms. However, given a gradient of increasing microbiome diversity, it would be valuable to understand when the selective advantage of the transition disappears. Other recent work, in microbiota-free mice, noted that when E. coli is a lone colonizer of the gut, it is consistently selected to increase metabolism of amino acids serine and threonine. A small increase in microbiome diversity (the addition of a single competing species) alters the evolutionary trajectory of E. coli substantially, instead favouring mutations associated with anaerobic metabolism\(^j\). This outcome suggests that bacteria may have low fidelity in metabolic function even within a single host generation\(^k\). Such a finding suggests host–microbial symbioses may not adhere to the idea of the ‘holobiont’ being a cohesive unit of selection\(^l\). This idea relies on high fidelity between partners\(^m\), which may easily be disrupted by changes to the surrounding microbial community.

If we can selectively drive the evolution of microorganisms and their communities, applications may improve on the already promising use of faecal microbiota transplants in medicine\(^n\), symbiont-mediated vector control\(^o\) and the manipulation of crop parasites\(^p\). There is, however, a pressing need to understand the long-term response of microbial communities to the engineering of symbions. Recently, theoretical models have treated virulence as a cost shared by all symbions coexisting in a host\(^q\). These models find that defence by a symbiont often drives reduced virulence across the microbial community (including in attacking parasites), an outcome dependent on the cost of defence being low and the shared cost of virulence also being low\(^r\). However, defensive microorganisms may also select for resistance mechanisms (for example, toxin production and inflammatory stimulation) in the parasites they protect against, causing collateral damage to hosts and driving increased parasite virulence\(^s\). This is akin to established predictions for co-infecting parasite species, whereby competition selects for increased virulence\(^t\). Promisingly though, and in line with some theory\(^u\), selection for reduced parasite virulence has been revealed in response to microbiome-mediated protection\(^v\). Others also report long-term efficacy of protection mechanisms despite an evolving pathosphere\(^w\).
symbiont-associated damage without limiting colonization\)\textsuperscript{,147} which reduces any negative impacts of the host–symbiont interaction. Evolving control mechanisms (for example, sanctions and rewards, and microbiome modulators)\textsuperscript{146,148} or acquiring symbiotic function from an alternative source (for example, symbiont switching and HGT)\textsuperscript{106} can also limit or cause a change in the position of the interaction along the continuum.

Resistance to symbiont infection is observed ubiquitously across evolving host–parasite associations\textsuperscript{149,150}. Mutations associated with membrane transporters in the bacterium \textit{Actinomyces odontolyticus} coincided with a reduction in the negative effects of its ectoparasite (\textit{Nanosynbacter lyticus})\textsuperscript{151}, perhaps indicating an adaptive host response to block resources to the ectoparasite or prevent its attachment\textsuperscript{151}. As host resistance and tolerance strategies can affect parasitic symbiont fitness, they can counter-adapt\textsuperscript{152,153}. This process may lead to a repeated back and forth along the continuum.

Hosts can also have key roles in restraining symbiont-driven shifts along the continuum. They may act to prevent the emergence of cheating symbionts, which exploit the benefits of host association without paying the cost of returning a benefit\textsuperscript{27,154}. Alternatively, hosts may maintain the association at a position optimal for their own fitness. Sanction and reward strategies, spatial segregation of symbionts and partner choice mechanisms have evolved to promote and maintain cooperation\textsuperscript{27,154,155}. For instance, legumes may sanction defective nitrogen-fixing bacteria by blocking resources to the respective root nodule\textsuperscript{32,154}, and plants reward helpful mycorrhizal fungi with extra carbohydrate\textsuperscript{156}. These mechanisms protect the host from investing in symbionts with net costs and avoid trajectories towards antagonism.

There is mounting theoretical and empirical evidence that many putative mutualisms may actually be a product of hosts exploiting symbionts\textsuperscript{27,157}. Interactions can benefit the host, but with no reciprocity to the symbiont whose fitness is markedly reduced within the walls of host confinement\textsuperscript{1}. These may be viewed as cases of inverted parasitism\textsuperscript{1}. The host is the parasite of its smaller guest. This phenomenon is exemplified by zoanthellae in which replication rates are severely compromised by host association\textsuperscript{1}, rising from 3 days outside of coral hosts\textsuperscript{157} to around 70 days within\textsuperscript{158}. Another example comes from \textit{Paramecium bursaria} and photosynthetic \textit{Chlorella} symbionts. \textit{Chlorella} species provide fixed carbon in return for organic nitrogen, but the host tightly controls symbiont density in response to light conditions, ensuring the best nutrient trade for itself\textsuperscript{159}. Control of the symbiont potentially occurs via digestion of \textit{Chlorella} cells\textsuperscript{160}. The host may win twofold, paying the workforce only when required and acquiring nutrition via digestion of surplus symbionts. The growth rate for \textit{Chlorella} remains consistently better outside the host\textsuperscript{159}, but inside, this symbiont avoids algal competitors\textsuperscript{161} and may be protected against its own parasites\textsuperscript{162}. Research on exploitation by hosts is in its infancy, with the greatest evidence coming from interactions with photosynthetic symbionts\textsuperscript{149,163}. Many questions remain, including the ubiquity of the phenomenon and whether some classes of symbiont are more vulnerable to exploitation than others.

Although considered relatively rare over evolutionary time, hosts may also eschew parasitic\textsuperscript{164} and mutualistic associations\textsuperscript{165}. Fleeing the infectious environment is one strategy. Spatiotemporal escape by asexual rotifers prevents them interacting with fungal parasites consistently over evolutionary time. By drying up and blowing away in the wind, these animals are protected from infection, which allows them to maintain their asexual reproductive strategy\textsuperscript{166}. Mutualistic associations can be abandoned via the recruitment of new symbionts\textsuperscript{100}. As the \textit{Hodgkinia} endosymbionts of cicadas teetered on the edge of genomic collapse, \textit{Ophiocordyceps} fungi (commonly parasites) began to take over the essential roles in amino acid synthesis for the host\textsuperscript{186}. Abandonment can also occur via exploitation of an alternative resource\textsuperscript{100}. For example, the evolution of carnivory in plants led to several plant species deserting arbuscular mycorrhizal fungal symbionts, as the plant now gains nutrients directly from prey\textsuperscript{186}. These cases chime with a growing debate over whether hosts can have the upper hand in symbioses, despite generally being the species that evolves more slowly (known as the Red King effect\textsuperscript{166,167}), exploiting and imprisoning their microorganisms to gain disproportionate control and benefit\textsuperscript{27,153,159,166}.

**Context-dependent shifts**

The outcome of many microbial interactions with hosts are context dependent\textsuperscript{1}. Both facultative and obligate symbioses can make shifts along the parasite–mutualist continuum that do not involve evolution, often occurring within a generation and driven by ecological change or opportunity (TABLE 5). Abiotic factors such as temperature\textsuperscript{166}, resource availability\textsuperscript{166}, environmental toxicity\textsuperscript{167} and the biotic composition of the surrounding community\textsuperscript{169} or host ontogeny\textsuperscript{2,170} can all affect the distribution of costs and benefits incurred by the host and microbial symbiont. The position on the continuum can also change if the microbial symbiont becomes infected with its own symbionts (for example, phages and mycoviruses)\textsuperscript{62,172}. Here, we focus on short-term disruptions to host–symbiont associations, but note that sustained alterations to context will feed back to evolutionary change for the interacting species.

Generally, theory predicts that nutrient-limited environments, or other harsh environments, can foster beneficial interactions between compatible players\textsuperscript{27,174} via such mechanisms as cross-protection and cross-feeding. This outcome has been substantiated by empirical work\textsuperscript{175–177}. For symbionts that have nutritional roles (for example, vitamin synthesis and nitrogen fixation), abundant resources can substantially undermine the net benefit gained by the host. The provisioning of mineral nitrogen from fertilizer erases the benefit \textit{Bradyrhizobium} symbionts provide to legume hosts (\textit{Lotus strigosus}) as this acquisition route is less energetically costly for the legume than its symbiont-fixed equivalent\textsuperscript{178}. Some hosts evade context-dependent costs by divesting themselves of associations when ecological conditions change, such as the phytoplankton that abandon their nitrogen-fixing cyanobacteria
when environmental nitrogen is abundant\(^{79}\). For host–parasite systems, there is no evidence for a one-way effect of nutrient availability to hosts on the harm caused by infection\(^{39}\). One study\(^{180}\) suggested that the level of parasite virulence in a given environment is likely the result of a balance between the effect of host nutrition on the immune system and on parasite resources.

Temperature can affect symbiont phenotypes\(^{95,182}\), which directly impact symbiont virulence or benefit, such as the regulation of toxin production\(^{183}\) or molecules required for nutrient scavenging\(^{184}\). Some obligate mutualists can constitute thermally ‘weak links’ for hosts, becoming non-functional or even lost from hosts outside adapted temperature ranges, which can have catastrophic consequences for host fitness\(^{185,186}\). Interactions can occur between abiotic and biotic factors. For instance, a 5 °C increase in temperature diminishes the longevity cost of hosting defensive symbiont differs across aphid genotypes\(^{187}\).

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Pathobionts provide an excellent example of context-dependent transitions from neutral to harmful agents\(^{188}\). In a host with a functional immune system and healthy microbiota, pathobionts can exist as commensals\(^{191-193}\). Pathobionts are well adapted to proliferate beyond their normal niche. During dysbiosis (for example, compromised immunity, disruption of the microbiota or introduction of medical devices such as catheters or surgical implants) pathobionts can cause disease in a wide variety of forms, from minor infections to more serious chronic or invasive disease\(^{194}\).

This ability to transition from harmless to harmful in different contexts makes pathobionts hard to place on the continuum. They are neither consistent parasites nor consistent commensals, with the state of the host generally determining their transition from one to the other.

### Stuck at the end of the line

At either end of the continuum lie the extremes of host-killing (or castration) and mutual dependence. What maintains an association here, and what is its future?
The ability to shift along the continuum for some parasitic microorganisms could depend on transmission route. Some infectious agents may stay virulent owing to a high degree of environmental transmission or a lack of reliance on hosts to transmit and propagate. The ‘curse of the Pharaoh’s’ hypothesis posits that microorganisms able to ‘sit and wait’ in the environment can be perpetual killers, whereas others suggest that traits that enable persistence in the environment will be traded off with virulence. There may also be constraints of the parasitic life cycle that prevent a transition. Microbial parasites that must lyse host cells to transmit (for example, lytic phages and Plasmodium species in mammals) or steal resources in a way that castrates the host (Pasteurella bacterial parasite infecting Daphnia magna) are systems in which transitions away from antagonism are unlikely.

At the opposing end of the continuum lie inherited, obligate endosymbionts, which often have nutritional roles. Although many of these associations are ancient and bestow mutual benefits, they can be risky, particularly for the endosymbiont. The genomes of these symbionts can gradually decay as transmission bottlenecks allow deleterious mutations to become fixed by genetic drift, and mutualistic bias towards deletions removes genes. Genomic decay can lead to extinction, unless heightened genetic and cellular support is provided by the host or other symbionts. For example, leafhoppers show gene expression patterns that appear tailored to the deficiencies of each of their endosymbionts’ highly degraded genomes. In rare cases, symbionts may transition to organelle status, notably achieved by mitochondria and plastids, but this does not guarantee shelter against further gene loss or extinction. Hosts may also avoid extinction alongside an endosymbiont by exploiting alternative nutritional resources or gaining new symbionts.

**Conclusions and future perspectives**

Plants and animals, including humans, are colonized by innumerable microorganisms. This observation has sparked a revolution in studying the impacts of those microorganisms on host biology and health. Many more examples of microbial evolution causing transitions across the parasite–mutualist continuum will emerge through further research using experimental evolution and investigating the microbiome in an evolutionary context. The potential evolution of species in the human microbiome from good to bad, and the degree to which beneficial interactions could be upset by microbiome perturbation, are of critical relevance for individuals vulnerable to infectious disease. In the future, such individuals may benefit from engineering of the microbiome or symbiont communities, via either direct genetic modifications to key transition loci in microbiome members, or exposure to selection sources with known outcomes. This approach has recently been achieved for honeybees, with the genetic modification of a core gut bacterium improving resistance to viral infection. These are exciting applications, but we must strive to understand the evolutionary consequences for the parasites targeted too.

More fundamentally, understanding causes of transitions will provide insight into the dynamics of how an organism’s biology and its community are shaped by microbial inhabitants. The ecological and evolutionary transitions of other species, as well as environmental change, can alter the scope for conflict in symbioses involving microorganisms. Interest has grown in thinking of host–microorganism symbioses as holobionts with highly aligned selective interests. Many associations may be also viewed in an ecological community context in which constant shifts occur back and forth on the parasite–mutualist continuum. The degree to which the host and symbiont, or both, have control over those shifts remains relatively unexplored. Research in the field has focused on the propensity of symbions to invade unwilling hosts or cheat reciprocal arrangements. Yet an exciting new avenue is emerging, one that is exposing hosts as exploiters and imposers of microbioms. The extent to which microorganisms are able to evolve to counter or take advantage of that exploitation is also unclear.

Moreover, environmental changes have the potential to substantially alter selection in symbiotic interactions. In addition to altering established symbioses, marked changes to abiotic variables can also move the boundaries of environmental constraint, fostering the evolution of new interactions on the continuum that were previously impossible or profitless. How will the collectively growing impact of humans affect the stability of beneficial associations and the emergence of parasites globally? This question is particularly timely given the COVID–19 pandemic. Undoubtedly, as environmental perturbations increase in magnitude and frequency, and as the use of antimicrobials grows, understanding the effects on the real-time evolution of host–symbiont interactions will become more and more valuable.

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