Microbial evolution and transitions along the parasite-mutualist continuum

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Abstract: Virtually all plants and animals, including humans, are homes for symbiotic microbes. These interactions can be neutral, harmful, or have beneficial effects for host organisms. However, growing evidence suggests that microbial symbionts can evolve rapidly, resulting in dramatic 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-microbe interactions are stuck at the end of the continuum. In addition to having biomedical consequences, understanding the dynamic life of microbes reveals how symbioses can shape an organism's biology and the entire community particularly in a changing world.

Introduction

Parasitic and mutualistic microbial symbioses exist widely in nature. These interactions occur when microbes (i.e., bacteria, fungi, viruses) take up residence in/on animals or plants, and cause damage or confer benefits to the host. Parasitic microbes (we include 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[1]. There is thus an emerging awareness that many putative mutualisms may even be hosts exploiting symbionts[2–4], in an interaction referred to as inverted parasitism[5].

The continuum

The designation of entities as 'parasite' or 'mutualist' implies a simple binary system whereby 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. They 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[6].

The concept of the parasite-mutualist continuum dates back several decades. An early discussion by Ewald[7] 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[8]. 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 microbes into parasites or mutualists

Microbes 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[9]. Furthermore, their capacity for plastic responses[10–12] and the dynamic nature of the communities microbes are nested and interact within[13,14], provide further routes for changing costs and benefits of association with hosts.

Free-living environmental microbes, that do not associate with hosts, were the progenitors for all symbiont diversity observed today[15]. Free-living microbes can evolve to be parasites or mutualists[16–21]. A new host-associated lifestyle often remains facultative for the microbe[22,23], but in some cases the

microbe evolves an obligate dependency on the host[24,25]. 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 (e.g. immune evasion, toxin production, nitrogen fixation, bioluminescence)[15,26]. Once associated with a host, symbiotic interactions can shift along the continuum (Fig. 1). 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[27,28]. Molecular phylogenetics corroborates this trajectory, showing that parasites have frequently served as progenitors for the independent descent of symbionts that now exhibit mutualistic traits[15,29]. Here, microbes shifting into novel host taxa is an important process, often forging novel associations on the continuum[30,31]. Transitions can also occur if a parasite's own selfish traits benefit a host as a by-product[27], or by hosts rewarding[32] or capturing[33] symbiont genotypes that confer benefits. Conversely, mutualisms can breakdown into parasitisms. This breakdown can occur due to the spread of cheater symbionts, which exploit the benefits of host association without paying the cost of returning a benefit[27,34]. However, shifts from mutualism to parasitism appear rare in nature[15,29,35]. More frequently, symbionts leave the host-association continuum by reverting to free-living environmental lifestyles, as demonstrated by actinobacteria abandoning ant hosts[15,36].

This review will discuss the evolutionary transitions of host-microbial symbioses along the parasite-mutualist continuum, the mechanisms underlying evolutionary changes, the selective pressures involved, and common empirical approaches to studying them (Box 1). We will also briefly discuss context-dependent transitions, and the consequences faced by microbes when their symbioses are constrained to the extreme ends of the continuum. We will moreover focus the review on eukaryotic host-microbe symbioses. We do however note that microbial interactions with mobile genetic elements (MGEs) can be analogous to symbioses (Box 2) given the ability of these elements to confer beneficial traits and cause harm to bacterial hosts[37,38].

Mechanisms of microbial evolution along the continuum

The gradual emergence of microbial mutualists from parasitic ancestors[15,29,31,39], and the rapid leaps in symbiont phenotypes observed in real time[40–44], 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 standing genetic variation[45,46], *de novo* mutations[40,43,47–49] and genome rearrangements[50–52]. Genome rearrangements

include inversions, duplications, translocations and gene loss[50,53,54] (for further discussion of gene loss, see later section 'Stuck at the end of the line'). Horizontal gene transfer (HGT) events, whereby genetic material moves between organisms in a manner other than vertically, are also important factors in microbial evolutionary transitions[42,55–58]. These events often involve mobile genetic elements (MGEs) – such as plasmids, transposons, insertion elements, and phages – coding for traits that are beneficial or harmful to hosts during their interaction.

Shifts between microbial parasitism and mutualism can involve selection on standing variation. Through experimental evolution of the bacterial symbiont *Parachlamydia acanthamoebae* and its protist host *Acanthamoeba* sp., Herrera et al.[46] 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 to result in movement along the continuum. In these cases, experiments are started by propagating a single clone in hosts. In King et al.[40], 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* started from a single clone[49]. Conversely, within the guts of old mice, mutations arising in clones of commensal *Escherichia coli* may have resulted in evolution towards pathogenicity[59]. Compared to 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 play a prominent role in transitions when symbionts have a low initial diversity upon colonisation. 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 played a profound role in *Yersinia pestis* becoming more virulent and adapting to new host species[50,60,61]. *Y. pestis* is the causative agent of plague in mammalian and arthropod hosts. It is thought to have diverged from its less harmful ancestor *Y. pseudotuberculosis* between 1,500-55,000 years ago[62,63]. Sequencing of isolates from the two species revealed that both HGT and insertion sequence-mediated genome rearrangements and deletions facilitated *Y. pestis* evolution[50,60,61]*.* The bacterium acquired

two plasmids, namely pMT1 and pPCP1, making it more virulent compared to its *Y. pseudotuberculosis* ancestor. The former plasmid carries the *ymt* gene encoding Yersinia murine toxin, required for the colonisation of the flea host[64,65], and the capsular antigen 'fraction 1', which inhibits phagocytosis[65,66]; these acquisitions contributed to 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 development to occur in the flea foregut[67]. 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[50,60,61]. 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 bacteriophages (mostly lytic, lambda-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[68]. 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 which have determined this bacterium's trajectory towards increased virulence in humans. One of these elements also encodes the major virulence factor LEE, the Locus of Enterocyte Effacement. It is responsible for bacterial attachment followed by development of the disease-causing effacing lesions in the intestine[69]. Lambda-like phages on the Sakai chromosome further encode for the destructive Shiga toxin, as well as genes 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 from *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[70–72]. For example, bacteriophage-driven HGT from the resident community can dictate the evolution of invading strains[73] and instigate change more rapidly than achievable by mutation accumulation[74]. HGT has played 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[20,29,54,58,65,75–80]. Yet, for symbionts nested within simple microbial communities (e.g. 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 transitions[40,49,59] 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 colonisation. When incorporating a host background with an ecologically relevant microbiota, HGT might be more dominant.

Drivers of evolution along the continuum

Ecological sources of selection can drive microbial symbiont evolution towards increasing host benefits (examples summarized in 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-microbe relationship due 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

Microbes frequently encounter novel host environments. They can jump across species boundaries or colonise hosts from pools of free-living environmental microbes. Novel infections can generate new diversity on the symbiosis continuum through divergence and speciation[81]. High profile cases of host shifts, such as the recent pandemic of SARS-CoV-2[82], highlight the potential for investigating evolutionary changes in virulence upon emergence[83–85]. New associations are often maladaptive for both host and parasite[86] and associations can move unpredictably on the continuum or 'burn-out'. This trajectory has been observed in emergences of avian influenza where case fatality rates can be high but human-to-human transmission is low[87].

Shifts between host species, possibly driven with HGT of virulence-associated genes, appears to have been important in the emergence of the Q fever parasite, *Coxiella burnetii*[30,88]. This proposed mutualist to parasite transition is a complex case for which the full evolutionary story remains unknown. Phylogenetic analysis however suggests this highly infectious microbe recently emerged from a clade of vertically-transmitted mutualistic endosymbionts of ticks[30]. *C. burnetii* may have evolved mechanisms to infect vertebrate cells, persist in the environment and be airborne-transmitted. These traits are unlikely to be found in the arthropod-restricted ancestors[30]. 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 was isolated after a patient suffered a wound from a tree branch, this serendipitous finding provided evidence that symbiont lineages emerged from environmental ancestors[31]. 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 microbe bears characteristics that can provide instant benefits. Microbes encoding functions of light generation, photosynthesis, nitrogen fixation or antimicrobials may provide such rapid benefits[15]. These characteristics may be remodelled (or act as preadaptations) for transitions in symbiosis[15]. Such repurposing may have occurred in the antifungal-producing *Burkholderia* symbionts associated with Lagriinae beetles. *Burkholderia* appear to have transitioned from plant parasite to insect mutualist. Here, secondary metabolites previously used as virulence factors against plants may have been repurposed for antifungal defence on beetle eggs[89]. Additional evidence comes from marine hosts, including within the bulbs of anglerfishes and the *Vibrio* filled light organs of bob tail squid. These hosts benefit from bioluminescent bacteria to lure prey and avoid predation respectively, and the symbionts often retain the capacity to live freely, or persist, in the environment[22,51].

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[7]. The degree of harm caused to hosts from infection is often framed by the virulence-transmission trade-off[90,91]. The relationship assumes that virulence – the reduction in host fitness caused by parasite infection – is costly to the parasite since host resources are needed for replication[92]. 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 the highest at intermediate virulence, which balances the costs of within-host replication and infectious period length[90]. This model is particularly pertinent for symbionts that rely on a mobile host for transmission (e.g. socially transmitted microbes). Those that do not (e.g. vector & water-borne microbes) can bypass trade-offs between virulence and transmission[91]. This conventional model goes some way to hypothesizing on general patterns of virulence, yet several extensions and alternatives have been suggested[93–95].

It has been suggested that mutualists may evolve from parasitic ancestors when the frequency of horizontal transmission routes is reduced or lost[7]. 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[7,90,96]. Mutualisms

involving symbiont inheritance are predicted to be stable on the continuum and unlikely to revert to parasitism[15,97]. But exclusive vertical transmission can endanger associations via genetic bottlenecking (see section '*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[15]. Many mutualisms involve horizontal transmission such as conjugative plasmids in bacterial populations[98] to the vast networks of mycorrhizae that improve plant productivity[99,100]. 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[101]. Many horizontally-transmitted microbial symbionts are obligate for host fitness[16,22,102], but many can be facultative[24] and confer costs in different environments.

Conversely, not all inherited microbes become mutualists[103]. *Wolbachia, Spiroplasma* and *Arsenophonus* are common inherited parasites that manipulate host reproduction, maximising resource allocation to the transmitting host sex (females) by feminizing hosts or killing their sons[104]. However, theory suggests that the spread of such reproductive parasites will be enhanced by the evolution of traits that benefit hosts[105]. A beneficial trait (i.e. defence) may even interact with a parasitic trait (i.e. reproductive manipulation) to completely exclude a natural enemy[105]. Indeed, cryptic benefits are now found in several systems[106,107], and there is evidence that some reproductive parasites may need to also transmit horizontally just to persist[108].

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 (see Tables 1 & 2). In a symbiosis between a jellyfish and the algae *Symbiodinium microadriaticum*, cooperative traits, including growth enhancement, were selected when transmission was restricted to heritable routes[109]. Such cooperative traits are fundamental for stable mutualisms, protecting against transitions to parasitism or abandonment events. In the reverse experiment, restriction of the algae to horizontal transmission selected for faster proliferation and dispersal (traits associated with parasitism), and declines in host fitness were detected[109]. Such findings are mirrored across terrestrial systems[46,110,111]. The common pill bug hosts a *Wolbachia* strain that feminises genetic males[112]. Blocking the typical vertical route, and mimicking horizontal transmission, saw systemic increases in *Wolbachia* density and a dramatic transition from a benign partner to a highly virulent one[110].

The Community

The drivers of transitions on the parasite-mutualist continuum can be complex and stem from the ecological and evolutionary movements of many different players. Defensive symbioses[113,114], whereby there are at least three interacting species (host, defensive symbiont, and attacking enemy) are 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[115,116]. Coinfections in hosts can also influence transitions in the symbiosis by providing new phenotypes via HGT of genetic material (e.g. symbiosis islands, plasmids, phage)[78,80,114,117].

The impact of community complexity is demonstrated by the bacterium *Hamiltonella defensa* and its lysogenic bacteriophage, APSE. This association protects host aphids against parasitoid wasps[118,119] (Fig. 2). Here, 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[120]. The mechanism of protection (toxin production) hinged on the initial lateral transfer of bacteriophage from a coinfecting symbiont[117,121]. Subsequent loss of the bacteriophage can move the interaction between *H. defensa* and aphids back towards parasitism[122]. Theory[105,116], experimental evolution[40] and field studies[123] have captured how microbes, 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[40]. In the parasite's absence, the gut bacterium did not emerge as a microbial line of host defence.

Additional symbionts, with previously unknown effects, are increasingly identified even in iconic 'two-player' symbioses, such as in corals[124] and lichens[125,126]. 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 whole. Members of the microbiota compete and cooperate in a myriad of ways[127], influencing the virulence of one another via processes such as the suppression of public goods[128] or the facilitation of biofilm formation[129] and epithelial translocation[130]. The passage of *Candida albicans* in mice lacking gut microbiota has highlighted the role of communities in determining fates on the parasite-mutualist continuum. In the absence of gut microbiota, *C. albicans* mutants emerge that are defective in hyphal formation, no longer requiring it for competition against other microbiota members. When compared to 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[43]. This transition from pathobiont to conditional mutualist here appears to hinge on the absence of competing microbes. 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 coloniser of the gut, it is consistently selected to increase metabolism of amino acids serine and threonine. One small increase in microbiome diversity (the addition of a single competing species) alters the evolutionary trajectory of *E. coli* significantly, instead favouring mutations associated with anaerobic metabolism[131]. This outcome suggests microbes may have low fidelity in metabolic function even within a single host generation[132]. Such a finding suggests host-microbe symbioses may not adhere to the idea of the 'holobiont' being a cohesive unit of selection[133]. This idea relies on high fidelity between partners[134] which may be easily disrupted by changes to the surrounding microbial community.

If we can selectively drive the evolution of microbes and their communities, applications may improve on the already promising use of faecal transplants in medicine[135], symbiont-mediated vector control[136,137], and the manipulation of crop parasites[42]. There is however a pressing need to understand the long-term response of microbial communities to the engineering of symbionts. Recently, theoretical models have treated virulence as a cost shared by all symbionts coexisting in a host[138,139]. These models find that defence by a symbiont often drives reduced virulence across the microbial community (inc. in attacking parasites), an outcome dependent on the cost of defence being low and the shared cost of virulence also being low[139]. However, defensive microbes may also select for resistance mechanisms (e.g. toxin production, inflammatory stimulation) in the parasites they protect against, causing collateral damage to hosts and driving increased parasite virulence[140]. This is akin to established predictions for coinfecting parasite species, whereby competition selects for increased virulence[141–143]. Promisingly though, and in line with some theory[138,139], selection for reduced parasite virulence has been revealed in response to microbe-mediated protection[144]. Others also report long-term efficacy of protection mechanisms despite an evolving pathosphere[145].

Host control

Beyond microbial symbiont evolution, hosts can affect the position of the symbiosis[146]. Hosts can be resistant (i.e. reducing symbiont colonisation) and tolerant (i.e. coping with symbiont-associated damage without limiting colonisation)[147] which reduce any negative impacts of the host-symbiont interaction. Evolving control mechanisms (e.g., sanctions and rewards, microbiome modulators)[146,148], or acquiring symbiotic function from an alternative source (e.g. symbiont switching, HGT)[100] can also limit or cause a change in position of the interaction along the continuum.

Resistance to symbiont infection is observed ubiquitously across evolving host-parasite associations[149,150]. Mutations associated with membrane transporters in the bacterium *Actinomyces odontolyticus* coincided with a reduction in the negative effects of its ectoparasite (*Nanosynbacter*

lyticus)[151], perhaps indicating an adaptive host response to block resources to the ectoparasite or prevent its attachment[151]. As host resistance and tolerance strategies can impact parasitic symbiont fitness, they can counter-adapt[152,153]. This process may lead to a repeated back and forth on the continuum.

Hosts can also have key roles in restraining symbiont-driven shifts on 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[27,34]. Alternatively, hosts may maintain the association at a position optimum for their own fitness. Sanction and reward strategies, spatial segregation of symbionts and partner choice mechanisms have evolved to promote and maintain cooperation[27,154,155]. For instance, legumes may sanction defective N-fixing bacteria by blocking resources to the respective root nodule[32,154], and plants reward helpful mycorrhizal fungi with extra carbohydrate[156]. These mechanisms protect the host from investing in symbionts with net costs, thus avoiding trajectories towards antagonism.

There is mounting theoretical and empirical evidence that many putative mutualisms may actually be a product of hosts exploiting symbionts[2–4,33]. Interactions can benefit the host, but with no reciprocity to the symbiont whose fitness is markedly reduced within the walls of host confinement[1]. These may be viewed as cases of 'inverted parasitism'[5]. The host is the parasite of its smaller guest. This phenomenon is exemplified by Zooxanthellae where replication rates are severely compromised by host-association[4], rising from 3 days outside of coral hosts[157] to around 70 days within[158]. Another example comes from Paramecium and photosynthetic *Chlorella* symbionts. *Chlorella* provides 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[159]. Control of the symbiont potentially occurs via digestion of *Chlorella* cells[160]. The host may win two-fold, paying the work force only when required and acquiring nutrition via digestion of surplus symbionts. The growth rate for *Chlorella* remains consistently better outside of the host[159], but inside, this symbiont avoids algal competitors[161] and may be protected against its own parasites[162]. Research on exploitation by hosts is in its infancy, with the greatest evidence coming from interactions with photosynthetic symbionts[4,159,163]. Many questions remain, including the ubiquity of the phenomenon and whether some classes of symbionts are more vulnerable to exploitation than others.

Although considered relatively rare over evolutionary time, hosts may also eschew parasitic[164] and mutualistic associations[100]. 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

401 them to maintain their asexual reproductive strategy[164]. Mutualistic associations can be abandoned via
402 the recruitment of new symbionts[100]. As the *Hodgkinia* endosymbiont of cicadas teetered on the edge
403 of genomic collapse *Ophiocordyceps* fungi (commonly parasites) began to take over the essential
404 roles in amino acid synthesis for the host[165]. Abandonment can also occur via exploitation of an
405 alternative resource[100]. For example, the evolution of carnivory in plants led to several plant species
406 deserting arbuscular mycorrhizal fungal symbionts, as the plant now gains nutrients directly from
407 prey[100]. These cases chime with a growing debate over whether hosts can have the upper hand in
408 symbioses, despite generally being the slower evolving species (known as the Red King Effect[166,167]),
409 exploiting and imprisoning their microbes to gain disproportionate control and benefit[2–4,33,159,168].
410
411
412 **Context-dependent shifts**
413
414 The outcome of many microbe interactions with hosts are context-dependent[14]. Both facultative and
415 obligate symbioses can make shifts along the parasite-mutualist continuum that do not involve
416 evolution, often occurring within a generation and driven by ecological change or opportunity (Table
417 3). Abiotic factors such as temperature[169], resource availability[170], environmental toxicity[171], and the
418 biotic composition of the surrounding community[119] or host ontogeny[172,173] can all disrupt the
419 distribution of costs and benefits incurred by the host and microbial symbiont. The position on the
420 continuum can also change if it the microbial symbiont becomes infected with its own symbionts (e.g.
421 bacteriophages, mycoviruses)[42,122]. Here, we focus on short term disruptions to host-symbiont
422 associations, but note that sustained alterations to context will feedback to evolutionary change for the
423 interacting species.
424
425 Generally, theory predicts that nutrient-limited environments, or other harsh environments, can foster
426 beneficial interactions between compatible players[27,174], via mechanisms such as cross-protection and
427 cross-feeding. This outcome has been substantiated by empirical work[175–177]. For symbionts that play
428 nutritional roles (e.g. vitamin synthesis, nitrogen fixation), abundant resources can significantly
429 undermine the net benefit gained by the host. The provisioning of mineral nitrogen from fertiliser
430 erases the benefit *Bradyrhizobium* symbionts provide to legume hosts (*Lotus strigosusas*) as this
431 acquisition route is less energetically costly for the legume than its symbiont fixed equivalent[178]. Some
432 hosts evade context-dependent costs by divesting of associations when ecological conditions change,
433 such as the phytoplankton that abandon their nitrogen fixing cyanobacteria when environmental
434 nitrogen is abundant[179]. For host-parasite systems, there is no evidence for a one-way effect of
435 nutrient availability to hosts on the harm caused by infection[180]. Pike et al. (2019)[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[181 182] 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 *Hamiltonella*-mediated defence against parasitoids[187,188]. This temperature-dependent reduction in defence may be ameliorated if coinfection with an additional bacterium, known as pea aphid X-type symbiont, occurs[187].

In other cases, community composition alone can temporarily cause transitions. Defensive symbioses present a clear demonstration of community context-dependent shifts, whereby benefits to the host are contingent on the presence of an enemy species[113,114]. In the absence of the enemy, the host pays the cost with no detectable benefit, and the association moves towards one that is a parasitic[114,189]. Infection of a symbiont with its own symbionts (i.e. hyperparasitism[190]) can also generate transitions. Recent work found that the devastating effects of a fungal parasite on rapeseed crop are significantly reduced if the fungus becomes infected with mycovirus SsHADV-1[42]. Presence of the mycovirus appeared to affect the expression of a suite of both fungal and crop genes, including those encoding plant cell-wall degrading enzymes and crop signaling pathways[42].

Pathobionts provide an excellent example of context-dependent transitions from neutral to harmful agents[191]. In a host with a functional immune system and healthy microbiome, pathobionts can exist as commensals[191–193]. Pathobionts are well adapted to proliferate beyond their normal niche. During dysbiosis (e.g. immune compromisation, 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 lies 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 microbes could depend on transmission route. Some infectious agents may stay hyper-virulent due to a high degree of environmental transmission, or a lack of reliance on hosts to transmit and propagate. The 'Curse of the Pharoah' hypothesis[195] posits that microbes able to 'sit and wait' in the environment can be perpetual killers, whilst others suggest traits that enable persistence in the environment will be traded-off with virulence[196]. There may also be constraints of the parasitic life-cycle that prevent a transition. Microbial parasites that must blow apart host cells to transmit (e.g. lytic bacteriophages, *Plasmodium* in mammals) or steal resources in a way that castrates the host (*Pasteuria* in *Daphnia* invertebrate hosts[197]) 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. While many of these associations are ancient and forge mutual benefit, they can be risky, particularly for the endosymbiont[3,53,198]. The genomes of these symbionts can gradually decay as transmission bottlenecks allow deleterious mutations to become fixed by genetic drift, and mutational biases toward deletions removes genes[199–202]. Genomic decay can lead to extinction, unless heightened genetic and cellular support is provided by the host[203] or other symbionts[78,204,205]. For example, leafhoppers show gene expression patterns that appear tailored to the deficiencies of each of their endosymbionts highly degraded genome[203]. In rare cases, symbionts may transition to organelle status[206], notoriously achieved by mitochondria and plastids, but this does not guarantee shelter against further gene loss or extinction[207,208]. Hosts may also avoid extinction alongside an endosymbiont by exploiting alternative nutritional resources or gaining new symbionts[158,159].

Conclusions and future perspectives

Plants and animals, including humans, are colonised by innumerable microbes. This observation has sparked a revolution in studying the impacts of those microbes 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[209,210], and the degree to which beneficial interactions could be upset by microbiome perturbation[211], are of clinical relevance for patients vulnerable to infectious disease. In the future, such patients may benefit from the engineering of microbiome/symbiont communities, either via 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 honey bees, with the genetic modification of a core gut bacterium improving resistance to viral infection[212]. 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 moves of other species, as well as environmental change, can alter the scope for conflict in symbioses involving microbes. Interest has grown in thinking of host-microbe symbioses as 'holobionts' with highly aligned selective interests[134]. Many associations may be better viewed in an ecological community context[13,146] 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 symbionts to invade unwilling hosts or cheat reciprocal arrangements. Yet an exciting new avenue is emerging, one that is exposing hosts as exploiters and imprisoners of microbes[33,198]. The extent to which microbes 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[213]. 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 impact the stability of beneficial associations and the emergence of parasites globally? (e.g. see Kiers et al.[214] and Lafferty[215]). This question is particularly timely given the coronavirus pandemic. Undoubtedly, as environmental perturbations increase in magnitude and frequency, and whilst the use of antimicrobials grows, understanding the effects on the real-time evolution of host-microbe symbiotic interactions will become more and more valuable.

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Glossary

Symbiosis: an association between two dissimilar organisms that have some degree of physical association and that is potentially long lasting, regardless of the implications for either organisms fitness.

Parasitism: an antagonistic symbiotic relationship in which one species is harmed, whilst the other benefits.

Mutualism: a symbiotic relationship in which both interacting species benefit, or are perceived to benefit. Benefit is often only confirmed empirically for the host.

Commensalism: an interaction in which the symbiont benefits, but has no measurable fitness effect on the host organism.

Inverted parasitism: an interaction where the classically viewed host exploits its smaller symbiont, implementing a fitness cost to the symbiont.

Free-living: a microbial lifestyle not dependent on association with a host for long term survival and replication, this is the ancestral state of all symbionts.

Defensive/protective symbiosis: an interaction in which the symbiont protects the host (via direct or indirect mechanisms) against natural enemies, such as microbial parasites and eukaryotic parasitoids.

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

Pathobiont: any organism that can cause harm to its host, but normally lives as a harmless symbiont.

Virulence: the damage caused to the host due to infection by a parasite, often measured as a reduction in host fitness.

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

Table 1 – Studies reporting evolutionary transitions of microbial symbioses towards the mutualism end of the continuum. These transitions involve reduction in virulence or increased benefit of the relationship to hosts over time.

Transition[a]	Host	Symbiont	Association	Condition	Mechanism / Evidence	Approach	References
P→P(-)	Ciliate *Paramecium caudatum*	*Holospora undulata*	Mixed mode transmitted parasite	Host demography allows high-growth	VT frequency increased relative to HT	Experimental	Magalon et al., 2010[216]
P→P(-)	*Actinomyces odontolyticus*	*Nanosynbacter lyticus* (TM7x)	Epibiont parasite	Naïve host & co-culture passage	Host susceptibility reduced, possibly by resource flows or binding sites disruption	Experimental	Bor et al., 2018[151]
P→P(-)	*Escherichia coli*	F1 phage	Parasitic phage	VT only	Benevolent variants favoured	Experimental	Bull et al., 1991[217]
P→P(-)	Nematode *(Caenorhabditis elegans)*	*Serratia marcescens*	Parasite	Coevolution over 20 gens	Increased host fecundity	Experimental	Gibson et al., 2015[218]
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	Alves et al., 2019[84] Kerr, 2012[85]

P→P(-)	Nematode *(Caenorhabditis elegans)*	*Staphylococcus aureus*	Parasite	Pathogen coevolved with defensive microbe	Siderophores production reduced	Experimental	Ford et al., 2016[144]
P→P(-)	Mouse *(Mus musculus)*	Friend virus (FV)	Parasite	Heterogenity in host resistance	Resistant hosts drove parasite specialisation, reduced mean virulence across host population	Experimental	Kubinak & Potts, 2013[219]
P→P(-)	Diamond-back moth *(Plutella xylostella)*	*Enterobacter cloacae*	Defensive microbe	Selection host protection	Complex trade-offs identified	Experimental	Matthews et al., 2019[220]
P→P(-)	Barley *(Hordeum vulgare)*	*Barley stripe mosaic virus* (BSMV)	Parasite	VT only	40% reduction in virulence	Experimental	Stewart et al.,2005 [111]
P→C	Nematode *(Caenorhabditis elegans)*	*Pseudomonas aeruginosa*	Gut parasite	Serial passage	Mutation in global regulator *lasR* and polymerase gene RpoB.	Experimental	Jansen et al., 2015[49]
P→C	Legume *(Mimosa pudica)*	*Ralstonia solanacearum* & *Rhizobial* plasmid[b]	Root nodulation	HGT & selection from emergent nodules	T3SS (*hrcV*) & master virulence regulator (*hrpG*) inactivated	Experimental	Marchetti et al., 2010[221]

FL/P→M	Squid *(Euprymna scolopes)*	*Vibrio*	Bioluminescence	N/A	Immune dampening of host NOS	Phylogenetic	Sachs et al., 2011[15] Ruby et al., 2005[222]
P→M	Nematode *(Caenorhabditis elegans)*	*Enterococcus faecalis*	Defensive microbe	Parasite driven selection	Increased antimicrobial - superoxide production	Experimental	King et al., 2016[40]
P→M	Mouse *(Mus musculus)*	*Candida albicans*	Gut symbiont	Gut microbiota absent	Filamentation loss, increased cytokine response, host-protection against infection	Experimental	Tso et al., 2018[43]
P→M	Fruit fly *(Drosophila simulans)*	*Wolbachia*	Reproductive parasite	VT & reproductive manipulation	Fecundity benefit over uninfected hosts	Experimental, field sampling	Weeks et al., 2007[123]
P→M	Cicadas *(Cicadoidea* spp*.)*	*Ophiocordyceps* fungi	Nutritional	Genomic decay of existing symbiont	Took over amino acid synthesis	Phylogenetic, field Sampling	Matsuura et al., 2018[165]
P→M	Pea aphid *(Acyrthosiphon pisum)*	*Hamiltonella defensa*	Defensive microbe	N/A	Putative parasite loci remain (T3SS & toxin homologs)	Comparititve genomic, phylogenetic	Degnan et al., 2009[121]

P→M	Insect spp.	*Sodalis*-allied	Insect endosymbionts	N/A	Mutualistic lineages stemmed from ancestors of putative parasitic strain	Phylogenetic	Clayton et al., 2012[31]
P→M	Lagriinae *Beetles*	*Burkholderia*	Antimicrobial producer	Host shift	Metabolite repurposed for insect defence	Phylogentic, experimental	Flórez et al., 2017[89]
P→M	*Arabidopsis thaliana*	*Pseudomonas protegens*	Rhizosphere associated	Low carbon forces dependence on host	Mutation in GacS/GacA TCS. Heightened competitiveness for host exudates	Experimental	Li et al., 2020[47]
P→M	*Amoebae proteus*	*Legionella*-like X-bacteria	Growth benefit	Association over 200 host generations	Evolved mutual dependence, altered host gene expression	Experimental	Jeon et al., 2004[223]
P→M	*Escherichia coli*	Cryptic prophage	Permanent host genome integration	Long-term coevolution	Increased host resistance to environmental stress	Experimental	Wang et al., 2010[224]
P→M	*Escherichia coli*	F1 phage	Parasitic phage	Serial passage	Enhanced growth rate & resistance to superinfection	Experimental	Bull & Molineux, 1992[225]

P→M	*Escherichia coli*	M13 phage	Growth inhibition	HT restricted	Growth benefit	Experimental	Shapiro et al., 2018[41]
P→M	*Pseudomonas fluorescens*	Mega-plasmid pQBR103	Mercury resistance	Mercury gradient	Host compensated by *gacA*/*gacS* TCS disruption, alleviated translational cost.	Experimental	Harrison et al., 2015[44]
FL / P→M	Stink bugs *(Pentatomidae spp.)*	*Burkholderia*	Gut symbiont	Unknown	Colonisation of midgut crypts	Phylogenetic, field sampling	Sachs, 2011[15] Kikuchi 2011[226] Kikuchi et al., 2007[227]
P / C→M	Bed bug *(Cimex lectularius)*	*Wolbachia*	Nutritional	Coinfection hypothesised	HGT of biotin operon	Experimental, genomic	Nikoh et al., 2014[80]
C→M	Squid *(Euprymna scolopes*	*Vibrio*	Bioluminescence	Host choice	Mutation in signalling protein gene (BinK), protected against host immune cells and chemicals	Experimental	Pankey et al., 2017[48]
M→M(+)	Jelly fish *(Cassiopea xamachana)*	Algae *(Symbiodiniium microadriaticum)*	Photosynthate provisioning	VT only	Host growth enhanced	Experimental	Sachs & Wilcox, 2006[109]
M→M(+)	*Escherichia coli*	M13 phage	Growth benefit	Transmission opport	Greatest benefit when VT & HT allowed	Experimental	Shapiro et al., 2016[228]

	unity
	varied

[a] Symbiont phenotype: Parasitism (P), Mutualism (M), Commensalism (C), Free-living (FL), (-) reduced, (+) elevated e.g. M → M (+) = indicates transition toward increased benefit to host

HT = horizontal transmission, VT = vertical transmission

N/A – specific drivers of transition unaccounted for due to timescale

636 **Table 2 –** Studies reporting evolutionary transitions of microbial symbioses towards the parasitism end
637 of the continuum. These transitions involve increased virulence or reduced benefit of the symbiotic
638 relationship to hosts over time.
639

Transition	Host	Symbiont	Association	Condition	Mechanism / Evidence	Approach	References
M→M(-)	Legume *(Ensifer medicae at)*	Rhizobia	N-fixing	Host choice blocked	Cheater strains favoured	Experimental	Porter & Simms, 2014[229]
M→M(-)	Legume (*Trifolium* spp.)	*Rhizobia*	N-fixing	Elevated N	Reduced cooperation under high nitrogen	Experimental	Weese et al., 2015[230]
M→P	Vertebrate spp.	*Coxiella burnetii*	Intracellular parasite	Host shift	HGT of virulence assoc. genes suggested	Phylogenetic	Duron et al., 2015[30]
M→P	Jelly fish *(Cassiopea xamachana)*	Algae *(Symbiodinium microadriaticum)*	Photosynthate provisioning	HT only	Greater proliferation in host & dispersal rates.	Experimental	Sachs & Wilcox, 2006[109]
M→P	Plant spp.	*Agrobacterium* spp.	Plant parasite	N/A	HGT of virulence loci	Phylogenetic	Sachs et al., 2014[29] Slater et al., 2009[231]
M→P	Plant spp.	*Pseudomonas syringae*	Plant parasite	N/A	HGT of HopZ T3SS effectors	Phylogenetic	Sachs et al., 2014[29] Ma et al., 2006[79]
M→P	*Escherichia coli*	M13 phage	Growth benefit	Host background	Parasitic when shifted to host ancestor	Experimental	Shapiro et al., 2016[228]

M→P	*Escherichia coli*	*F1 phage*	Parasitic phage	HT allowed	Antagonistic variants favoured	Experimental	Bull et al., 1991[217]
C→P	Pill bug *(Armadillidium vulgare)*	*Wolbachia*	VT endosymbiont	HT only	Titre increased in non-germline associated tissue	Experimental	Le Clec'h et al., 2017[110]
C→P	*In vitro* immune envrionment	*Escherichia coli*	Commensal strain	Macrophage pressure	Heightened macrophage evasion & delayed phagosome maturation, via TE insertion	Experimental	Proença et al., 2017[232]
C→P	*Arabidopsis thaliana*	*Pseudomonas fluorescens* species complex	Rhizophere associated	N/A	Gain of putative pathogenicity island (LPQ)	Comparitive genomics, phylogentic	Melnyk et al., 2019[39]
C→P	Plant spp.	*Rhodococcus* spp.	Plant-associated	N/A	Gain of virulence plasmid (pFID188), host growth inhibition	Experimental, comparitive genomics, phylogentic	Savory et al., 2017[58]
P→P(+)	Plant spp.	*Xanthomonadaceae* spp.	Phytopathogen	N/A	Gain of hydrolase gene (*cbsA*). Localised parasite become systemic.	Comparitive genomics, phylogentic	Gluck-Thaler et al., 2020[54]

P→P(+)	Barley *(Hordeum vulgare)*	*Barley stripe mosaic virus (BSMV)*	Parasite	HT only	Increased virulence, independent of titre	Experimental	Stewart et al.,2005[111]
P→P (+)	House finch (*Haemorhous mexicanus*)	*Mycoplasma gallisepticum*	Emerging parasite	Adaptation to novel host	Linear increase in virulence since shift	Natural sampling	Tardy et al., 2019[83]
P→P (+)	Mouse *(Mus musculus)*	*Cryptococcus neoformans*	Opportunistic parasite	Serial passage	Increased expression of iron reductase & host mortality	Experimental	Hu et al., 2014[233]
P→P (+)	Amoebae (*Acanthamoeba* sp.)	*Parachlamydia acanthamoebae*	Obligate intracellular symbiont	HT only	Enhanced infectivity & virulence, T3SS upregulated	Experimental	Herrera et al., 2020[46]
P→P(+)	Mammal spp.	*Yersinia pestis*	Enteric parasite	N/A	HGT of plasmids (pMT1 & pPCP1), increased transmissibility by fleas & virulence to mammals	Genomic	Lindler et al., 1998[65]

[a] Symbiont phenotype: Parasitism (P), Mutualism (M), Commensalism (C), Free-living (FL), (-) reduced, (+) elevated e.g. P→ P (+) = indicates transition toward increased parasitism

HT = horizontal transmission, VT = vertical transmission

N/A = specific drivers of transition unaccounted for due to timescale

643

644 **Table 3** – Examples of context-dependent transitions of microbial symbioses along the mutualist-

645 parasite continuum

646

Context [a]	**Species example**	**Transition**
Ontogeny	Queen conch– Symbiodinium[172]	Growth and survival benefit at larval stage, but photosynthetic activity of Symbiodinium at adult stage potentially limited due to shell cover (M→ P)
Host genotype	Aphid spp. – *Hamiltonella* defensa[189] [b]	The longevity cost of hosting defensive symbiont differs across aphid genotype
Temperature	Scleractinian coral – Symbiodinium[169]	Elevated temperature significantly reduced net primary productivity of coral, but no cost to Symbiodinium detected (M→P)
Metabolic	*Chlamydomonas reinhardtii* - *Saccharomyces cerevisiae*[170]	Mutualism between microbes occurs only in CO_2 restricted environment (FL→M)
Coinfecting microbes / Microbiome	Aphids – *Hamiltonella defensa* – *Serratia symbiotica*[119]	Coinfection provides additive benefit, enhancing host resistance to parasitoid wasps (M→M+)
Symbiont passengers (e.g, bacteriophages, mycoviruses)	Brassica crop - *Sclerotinia sclerotiorum* – *mycovirus*[42]	Mycovirus converts a fungal parasite into a crop enhancer (P→M)
Enemy presence	Drosophila - Wolbachia[234] [c]	*Wolbachia* protects against viruses. In absence of viral threat, host pays the cost of significantly curtailed lifespan (M→P)
Environmental toxicity	*Pseudomonas fluorescens* - Mercury plasmid[171]	Fitness effects of plasmid carriage varied with environmental mercury levels

Host switch	Nematodes - Xenorhabdus[235]	Mutualistic strains are harmful in non-native host (M→P)
Light	Hydra – Chlorella algae[236]	Under dark conditions Chlorella is costly, indicated by a growth disadvantage over uninfected hydra (M→P)

[a]Contextual variables can affect both host & symbiont processes independently, which may affect transitions, [b] Interactions between host genotype, symbiont genotype and the environment (G X G X E) also operate here, [c] Reproductive manipulation phenotype operating

Parasitism (P), Mutualism (M), Free-living (FL), (-) reduced, (+) elevated e.g. M→ M (+) = indicates transition toward increased benefit for host

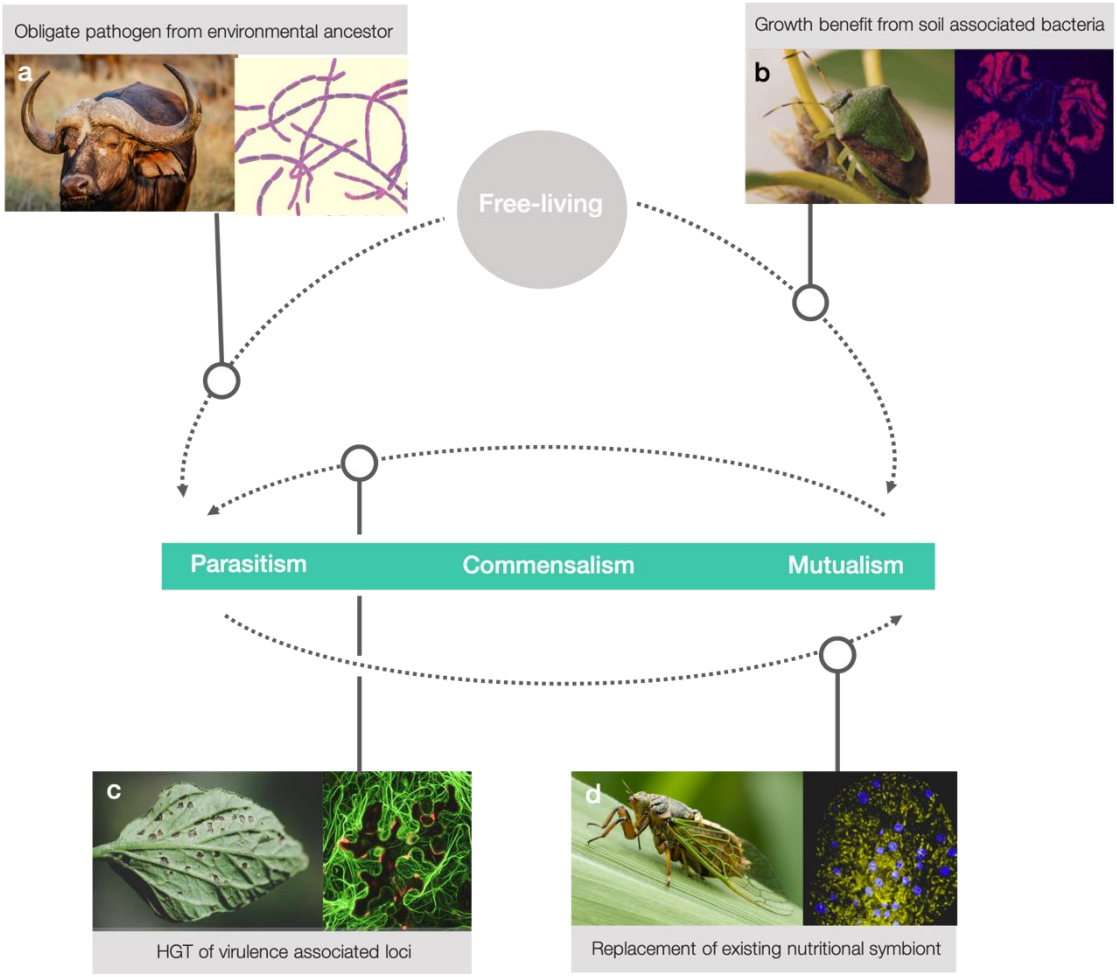

Figure 1 – Evolutionary transitions onto and along the parasite-mutualist continuum. Examples from nature of microbes transitioning from free-living to host-associated life styles include (a) the evolution of parasitic species in the *Bacillus cereus* group (e.g. agent of anthrax) from soil dwelling ancestors[237], and (b) environmental *Pantoea* bacteria evolving obligate mutualistic roles in stink bug growth and development[16]. Examples involving transitions along the continuum are (c) the widespread plant parasite *Pseudomonas syringae* likely evolving from mutualistic ancestors, driven by HGT of type-III secretion systems[29,79], and (d) entomopathogens taking over the metabolic role of an ancient and degraded endosymbiont in Cicadas[165]. Image credits: creative commons 4.0 (a), Takema Fukatsu[238] (b), Gerald Holmes, CC BY-NC 3.0 US (c, LHS), Kang et al., 2014[239], CC BY 4.0 (c, RHS), Yu Matsuura (d, LHS), Matsuura et al., 2018[165] (d, RHS)

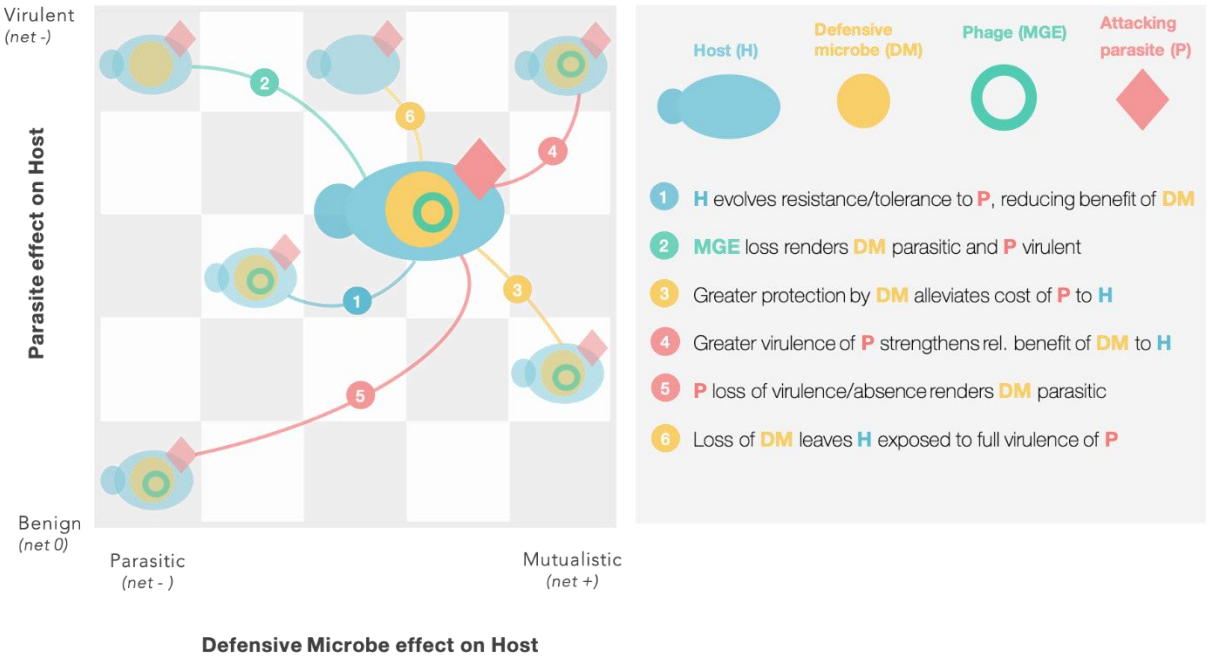

Figure 2 – Transitions in a community context. Defensive symbioses involve multiple species, including a host (H) and defensive microbes (DM) that protect against an attacking parasite[113] (P). Often hidden players exist within a DM, such as mobile genetic elements (e.g. phage/plasmids/TEs) that encode factors involved in the DM's protective function. In this community, the evolutionary and ecological moves (examples denoted by arrows) of each player can affect the relative position of another on the parasite-mutualist continuum. For example, if a mobile genetic element encodes key protective functions, then it's loss (2) will shift the DM's position towards parasitism (all cost and no benefit to host). Meanwhile, the costs of P to H will increase now that H is no longer protected by the DM and its mobile genetic element. Transitions here can also alter the coevolutionary patterns and processes between different players and species.

Box 1. Two common approaches to evaluating evolutionary transitions along the parasite-mutualist continuum

Phylogenetic inference

Challenges surround judging transitions in symbiosis as 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 help shed light on the evolutionary history of transitions on the host-microbe symbiosis continuum. Techniques such as ancestral state reconstruction and its extensions infer characteristics of ancestral taxa based on traits exhibited by extant descendents[240]. In this way, symbiotic phenotypes of ancestors (e.g. parasite, mutualist, commensal, 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[29]. Such approaches are heavily contingent on the quality of the underlying phylogenetic tree and reconstruction accuracy declines with increasing evolutionary time[240]. 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[15,29].

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 (e.g. *Caenorhabditis elegans*[241]*,* Paramecium[159]) 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[242].

In an evolution experiment, the source of selection can be hypothesized and be manipulated. For example, this approach could be used to determine whether the presence/absence of an enemy could affect the position of a defensive symbiosis along the continuum[40], as well as whether the evolution of the eukaryotic host, the microbial symbiont, or their coevolution was responsible for the shift[218]. 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[243]. 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/cost for each species, as well as to confirm phenotypic traits under selection. One caveat is that it might be less likely to observe increases in parasite virulence given the potential for breaking apart of the virulence-transmission trade-off at passage points[244].

Box 2. Mobile genetic elements as symbionts

Mobile genetic elements (MGEs) can cause genomic change in their microbial hosts. These changes can impact the position of the microbe-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 symbionts[37,38] 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 HGT[245], 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 killing by the toxin[246]. 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 time[58,247].

MGEs are not always maintained through natural selection, but also via genetic drift. The genome of *Wolbachia pipientis* wMel, an obligate intracellular symbiont of the fruit fly *Drosophila melanogaster*, is highly streamlined from extensive gene loss during adaptation to its host, but it is also overrun with MGEs[248]. Repeated population bottlenecks resulting in genetic drift and inefficient natural selection[248] likely contributes to the extensive maintenance of MGEs in this genome and those of other heritable symbionts[249]. These elements may have contributed to the substantial phenotypic diversity among *Wolbachia* strains, fundamentally shaping *Wolbachia* evolution[248]. 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 the *Wolbachia* genome.

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 translation cost[44]. Such compensatory evolution may also explain the persistence of context-dependent mutualisms in environments where they do not benefit hosts.

MGEs can also become 'immortalised' 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 immortalised in the genomes of organisms throughout the tree of life[250]. Bacterial chromosomes, for example, can contain as much as 20% bacteriophage DNA[251,252]. Once parasites to their hosts, these MGEs have infected the genomes of host organisms, maintained their stability as they co-evolve with their host (forcibly in some cases, e.g. toxin-antitoxin systems), and finally been irreversibly integrated into the genome. Integration can occur by accident during genome rearrangements, recombination, population bottlenecks and speciation events[248], or by natural selection because of a fitness benefit on which the host has become dependent[68]. The ubiquitous presence of vestigial viral DNA in the cells of all organisms[250] 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 other cases their maintenance has been a by-product of environmental conditions or population bottlenecks. They represent fascinating examples of entities which can be both effectors and subjects of evolutionary transitions along the parasite-mutualist continuum.

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