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Interspecific interactions among parasites in multiple infections

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Abstract

Individual hosts and populations frequently harbour multiple parasite species simultaneously. Despite their commonness, the consequences of interspecific interactions among parasites for determining infection outcomes are still poorly understood. We review and propose several expectations for multiple infections involving different species. We highlight that interspecific interactions affect the outcome of competition within hosts and that heterospecific parasites engage in co-transmission, gene exchange and reproductive interference. Studies specifically comparing intra- and interspecific coinfections and knowledge from community ecology may be instrumental to fully understand the consequences of interspecific multiple infections for parasite life-history ecology and evolution.

The diversity of multiple infections

Parasites are ubiquitous and host populations are often infected with more than one genotype [1,2]. As compared to single infections, **multiple infections** (see **Glossary**) in a host individual or population can have very different consequences for both host and parasite life-history and evolution. They can affect their virulence, as well as other traits such as transmission or competitive ability [3,4]. Coinfecting parasites can have very different taxonomic relationships: they can belong to the same species (e.g. two different strains of *Plasmodium chabaudi* in mice [5]) or to different species, which can be closely related (e.g. ectoparasitic mites *Tetranychus urticae* and *T. evansi* on tomato plants [6]) or from highly-differentiated guilds (e.g., *Plasmodium* or other microparasites and helminth worms in mice [7]). When parasites co-occur in a host population, interactions in the within-host environment are mostly described by **super-infections** or **co-infections** [8], although other possibilities exist [9]. Within hosts, the most common interaction among parasites may be competition for resources [10]. However, they can also engage in interference competition via the production of toxins [11], competition mediated by the immune system [12]; or they may instead facilitate each other (e.g. [13]). In addition, they can be co-transmitted among hosts with variable consequences for parasite evolution [14] (see **Table 1**).

This review aims to show the importance of being explicit about whether parasites in multiple infections belong to the same or a different species. We thus explore the scope for interspecific interactions among parasites in multiple infections to have specific impacts on hosts and parasites. First, we address factors that may promote the occurrence of interspecific multiple infections in host populations. We then address where interspecific interactions fit into the theory of the evolution of virulence. Next, we report how interspecific competition can be coupled with intraspecific competition to predict the outcome of coinfections within hosts. We subsequently discuss how genome integration among different species and their sexual interactions may affect host-parasite interactions. Finally, we address the role of interspecific interactions in maintaining host and parasite diversity.

Factors promoting the prevalence of multiple interspecific infections in host populations: co-occurrence, within-host interactions and co-transmission

Multiple infections comprising different parasite species are expected to affect virulence evolution (**Box 1**), but only if they are prevalent within a host population. This requires that parasites from different species co-occur in time and space and encounter one another in the same host population, and potentially in the same individual host. While coinfections involving several strains of the same parasite species may be due to shared life-history traits, this may not be the case for parasites of different species. Still, many factors may promote the co-occurrence of multiple parasite species in a host population.

Coinfections may occur simply because different parasite species have overlapping epidemics and high prevalence. A recent study found that this was the case in natural populations of *Daphnia magna* infected with different parasite species, suggesting that parasite interactions have little impact on the prevalence of coinfections [15]. However, in several systems, coinfection patterns cannot be predicted by single species distributions alone, as in plant viruses [16], rodent pathogen communities [17,18] or human malaria [19].

Non-random associations among parasites in multiple infections may result from within- or between-host interactions. Within the host, increased virulence due to coinfection may result in fewer coinfections than expected by single species/strain distributions [20]. Whether this is more or less likely under inter- or intraspecific interactions will hinge upon how these interactions affect parasite fitness (see **Box 1**). Within-host interactions also affect species composition in coinfections. For instance, the tomato infecting bacterium *Pantoea dispersa* is more resistant to invasion by another species, *P. protogens*, but only when it had evolved on that host plant (possibly due to within-host adaptation) [21]. Also, coinfection of the plant *Plantago lanceolata* with different *Podosphaera plantaginis* bacterial strains is more likely when their fitness is similar [22] and when particular bacterial species infect first [23]. Whether such interactions favour the co-occurrence of different parasite species will hinge upon their relative effect on parasites from the same or different species (see section 3).

Parasite interactions during between-host transmission may also affect the prevalence of coinfections. Indeed, parasite species are more likely to co-occur within the same individual host if they are **cotransmitted**. Some parasites of different species are obligately co-transmitted. For example, satellite nucleic acids are encapsidated by capsid proteins of their helper virus [24]. Also, several parasites are vectored by other pest/parasite species, as is the case of tomato spotted wilt virus, which relies on thrips for transmission. Thrips are important crop pests and co-transmission has been shown to be beneficial to both the virus and its vector, as the virus benefits by being transmitted, whereas thrips have higher fitness on virus-infected plants [25]. This pattern is true for many virus-vector systems [26]. In both these cases only one parasite relies on another for transmission. However, we are not aware of any examples where two horizontally transmitted parasites are both mandatorily cotransmitted.

Cotransmission may also emerge because parasites share the same transmission mode or route, such as certain sexually transmitted chronic diseases [27] or vertically transmitted symbionts [28]. Parasites that share the same vector species may also be cotransmitted, such as the arboviruses dengue, chikungunya and Zika, vectored by the mosquito *Aedes aegypti* [29] or plant viruses vectored by the whitefly *Bemisia tabaci* [30]. However, potential competitive interactions within the vector may hinder the successful transmission of all parasites to the next host [30]. Increased coinfection prevalence may also arise from transmission by the same vector but at different transmission events, especially if the prevalence of viruses and vectors is high [31].

Even if parasite transmission is independent, coinfections comprising different parasite species may be more likely to occur if parasites are more attracted, or infectious, to hosts harbouring another species of parasite. Immunosuppression of the host is one mechanism that might lead to increased coinfection [32]. In line with this, *Gammarus pulex* infected with the acanthocephalan *Pomphorhynchus laevis* are more susceptible to secondary infections [33]. Also, the spider mite *Tetranychus urticae* is more attracted to, and has higher fitness on tomato plants infected with tomato spotted wilt virus, possibly due to negative immune cross-talk preventing the host from mounting an effective immune response against spider mites [34].

Unboxing the black box: understanding the persistence of coinfections within the host.

The study of interactions among parasites within hosts has focused more on the outcomes of infections rather than on the underlying processes governing such outcomes [4,35]. This black-box approach has led theory to focus on either super-infections (e.g., [36]) or coinfections (e.g., [37]). These approaches do not account for the plethora of possible interactions among parasites, which has led to recent work claiming the need for a more mechanistic approach to the study of coinfections [9,38]. Indeed, it is only by understanding the mechanisms underlying parasite interactions that it may be possible to predict outcomes and potential parasite evolution in coinfections [39,40].

Coexistence theory (**Box 2**) emerges as a natural foundation to address coinfections mechanistically for several reasons. First, it accommodates different interactions among parasites, from apparent to interference competition [41,42] to facilitation [13]. Second, its recent extensions to network theory [43] allow incorporating interactions between parasites and hosts, which is key to establishing a solid link between multiple infections and the evolution of virulence. Finally, although coexistence theory is a general framework for community ecology, it predicts the exact same variety of outcomes as those predicted by frameworks specifically based on multiple infections ([9]; see **Box 2**).

A few highly controlled experiments have used coexistence theory in systems with parasites [6,44,45]. For instance, Fragata et al, (2022) [6] show that *T. evansi* generally excludes *T. urticae* except when the latter arrives first to a host plant and occupies *T. evansi's* preferred niche, in which case the two species are predicted to coexist in a coinfection scenario. This work highlights the importance and relevance of combining the fields of Host-Parasite Interactions with that of Coexistence Theory, by showing that the concepts of competitive exclusion and priority effects are relevant to multiple infections (**Box 2**). Yet, many potential avenues for future research remain to be uncovered. In particular, we lack understanding of how multiple infection outcomes, as predicted by coexistence theory, connect with emerging infection properties such as

155 virulence and parasite fitness (R_0, the number of new hosts that become infected from a
156 given infection in a susceptible population for microparasites or the number of infectious
157 stages produced per infection period for macroparasites, [46]). For instance, we could
158 hypothesise that virulence and stabilising effects might be negatively related because
159 the stronger the stabilising effect, the more parasites specialise on different resources
160 (niches), leading to fewer negative interactions among them, reducing selection for
161 increased growth, hence virulence.
162
163 **Reproductive interference among parasites**
164 When closely related species meet within a host, they may compete for resources but
165 also engage in costly interspecific sexual interactions, termed **reproductive**
166 **interference** [47]. Such interactions negatively affect the fitness of at least one of the
167 species involved through a wide range of possible underlying mechanisms, varying from
168 signal jamming and gamete wastage, to the production of inviable or sterile hybrids [47].
169 As for free-living organisms, the consequences of reproductive interference between
170 parasites may drastically differ from those of competition. As it promotes positive
171 frequency dependence (fitness being positively correlated with frequency in the
172 population), reproductive interference can increase the extinction risk of the rarer
173 species. It should thus preclude coexistence within hosts (as predicted for free-living
174 organisms at a local scale [48]). However, reproductive interference may also select for
175 behavioural avoidance of infected hosts [49], which may allow coexistence at the level
176 of the host population (i.e., regional coexistence for free-living organisms; e.g. [50]).
177 Additionally, it may act in combination with resource competition, in which case theory
178 predicts a reduction in the conditions allowing for coexistence [51]. This has been
179 recently validated experimentally in a system composed of two spider mite sister
180 species, *T. urticae* and *T. cinnabarinus*, infesting bean plants [52]. Yet, studies
181 investigating the interaction between reproductive interference and competition for food,
182 especially in host-parasite systems, remain rare. This is unfortunate, as such studies
183 are potentially highly relevant for sexually reproducing parasites, especially those that
184 share a niche within the host, such as reptile ticks [53], malaria parasites [54,55], and

platyhelminths in toads [56]. For instance, reproductive interference was suggested as the cause of spatial niche segregation in closely related helminth parasites [57].

Reproductive interference and competition may also affect the production of sexual stages in haemosporidian parasites such as *Plasmodium* [54,55]. Reproductive interference between parasite species can hamper sexual reproduction, which occurs in the vector for this parasite species [54,55]. This could select for higher asexual replication within the vertebrate host. Alternatively, interspecific competition may directly select for asexual growth in the vertebrate host, as observed in mixed strain infections [58]. Hence, both types of interaction (within-host competition and reproductive interference) may drive increased virulence but reduced or delayed transmission and possibly asynchrony in the production of sexual stages and sexual reproduction among parasite species (e.g. [55]).

Across species incorporation of genetic material

Some empirical studies have reported that parasites may incorporate DNA from other species, changing life-history traits and impacting epidemics. This can occur via hybridisation between closely related parasite species (reviewed in [59]) or the transfer of genetic material among more distant species. Indeed, hybrid parasites may have higher virulence (e.g. *Trypanosoma* [60]), transmission potential (e.g. *Leishmania*, [61]) and a greater host range than their parental species (e.g. *Schistosoma* [62]). Gene transfer of the virulence gene *ToxA* from the wheat pathogen *Stagonospora nodorum* into the non-pathogenic fungus *Pyrenophora tritici-repentis* leads to the production of pathogenicity-related toxins in the latter [63]. Horizontal gene transfer among plant species may have increased the adaptive potential of some *Orobranchaceae* parasitic plants to their hosts [64]. In addition, viruses with segmented genomes may exchange entire segments, a process called reassortment, even among distantly related genotypes (e.g. with > 50% nucleotide divergence [65]). Reassortment among avian influenza lineages with lineages circulating in human populations have been responsible for the past three human epidemics [66].These events of genetic exchange, although probably stochastic and/or rare, are noted since they confer a parasite with a fitness advantage not observed in the parental lineage. Whereas hybridisation or reassortment

is a likely by-product of coinfection by closely-related parasite species, gene transfers among more distant parasite species may be less prevalent, hence harder to foresee or identify after they occur. In any case, there is a large scope for genetic exchange during coinfections with interspecific parasites which can in turn affect important life-history traits, including virulence [59,63,67].

Impact of interspecific coinfections on host and parasite diversity

Infections with parasites from different species, by exposing hosts to a more variable environment, hold the potential to maintain genetic diversity in both parasite [68,69] and host [70,71] populations, via $G_P x G_P x G_H$ interactions. There is indeed evidence that parasitic traits may vary with the identity of the infecting species. For example, infection success of the fish eye flukes *Diplostomum pseudopathaceum* and *D. gasterostei* varies with the combination of the competing strains, increasing or decreasing by up to ~30% depending on initial dose [72]. Also, the outcome of competition between nematodes depends on the genotype of different species of *Xenorhabdus* bacterial symbionts and whether interactions are mediated via toxins (reduced growth and insect host mortality) [11]. Similarly, different population combinations of the ectoparasites *Tetranychus urticae* and *T. evansi* lead to higher or lower numbers of each species under coinfection *vs.* single infection [73]. Importantly, the traits observed in these multiple infections are not necessarily predictable from observations in the corresponding single infections [11,72,73]. Therefore, coinfections lead to variation in infection-related traits, which in turn may result in different selection pressures that hosts and parasites are exposed to, potentially maintaining variation in their populations.

Multiple infections may also modify coevolutionary dynamics and therefore host and parasite diversity. A recent theoretical study shows that coinfections can intensify or dampen fluctuating selection dynamics, depending on the fitness cost for the host [74]. This, in turn, may affect host diversity. Coinfections may also slow coevolutionary dynamics by making host-parasite interactions more diffuse (because not only between one host and one parasite, see [75]). This may diminish the diversification potential of this process [76,77]. Curiously, however, empirical data suggests otherwise, as

exposure of bacterial hosts (*Pseudomonas aeruginosa*) to more variable virus communities has no effect on within-population genetic diversity and leads to increased divergence among populations [78].

Concluding remarks: Future avenues for research

Interspecific multiple infections are expected to have multifarious consequences for interactions among hosts and parasites as well as for their evolution. Indeed, they have particularities that are not recapitulated in intraspecific interactions, particularly the need to account for the stabilising effect of niche differences and the potential role of reproductive interference. Still, our knowledge of the specific impact of interspecific multiple infections is as yet in its infancy. To obtain a comprehensive understanding of this impact, we here suggest a few future research avenues.

Arguably the most relevant knowledge gap is the prevalence of interspecific coinfections in natural populations. Currently, available information on this issue is scattered and mostly concentrated in a few model systems (e.g. [16-18,79]). We lack ecological knowledge on the relative proportion of coinfections by different parasite species compared to coinfections by conspecific parasites, and single infections (see **Outstanding Questions**). This knowledge is key to unravelling the forces shaping epidemiology and evolutionary processes in both hosts and parasites. Advances in genomic and metabarcoding approaches that will enable simultaneous identification of multiple parasite species may shed light on this.

Next, it is also key to identify the factors governing the prevalence of coinfections. One hypothesis drawn from coexistence theory is that the likelihood of coinfection depends on the phylogenetic distance among competing parasites [80]. If more distantly related parasites have diverged in their resource requirements and have resulted in reproductive barriers, then such stabilising niche differences may promote their coinfection. Conversely, if genetic changes have increased the competitive ability of one parasite over the other, then superinfection is expected, and coinfections would only occur among close relatives. Both outcomes have been documented in the literature on plant community ecology [81,82], but this information is largely missing for multiple infections. Using community ecology principles may be instrumental for a

deeper understanding of multiple infections. This was illustrated by Budischak et al (2018) [83] and Graham et al (2008) [7], who showed that outcomes of coinfection can be predicted based on whether interactions are bottom-up (competition for shared host resources) or top-down (mediated via host immune responses). Moreover, depending on the phylogenetic distance, interacting mechanisms among parasites may differ (see **Outstanding Questions**). For instance, closely-related parasites may interact more via competition [6] and/or reproductive interference [52] within the host, while an interaction more characteristic of distantly-related species may be via negative cross-talk between different branches of the host immune system (e.g., [7,84]). It should be noted that a recent meta-analysis across 424 species pairs (269 species) in free-living organisms found that functional similarity, rather than phylogenetic relatedness, predicted the strength of interspecific competition and thus may better explain patterns of community assembly [85]. Whether this is also true for parasites in coinfections remains to be tested (but see [7]). Such studies further understanding about general rules that can be applied to predict the outcomes of coinfections comprising different parasite species.

The phylogenetic distance or functional similarity between coinfecting parasites may also affect virulence (see **Outstanding Questions**). To address this issue, detailed comparisons between coinfections with conspecifics and heterospecifics are needed. Such a comparison has been made by Staves and Knell (2010) [86], who documented higher virulence on a wax moth when coinfected with different strains of the fungus *Metarhizium anisopliae*, but lower virulence when the coinfection included an entomopathogenic nematode [86]. More such examples are needed. Another related knowledge gap is the relative contribution of each parasite to the overall virulence of coinfections. Measuring this may be more feasible when parasites are from different species if symptoms are distinct and can be linked to the fitness of each parasite. This information is important to understand when virulence under coinfection is higher or lower compared to the sum of the virulence of each parasite separately [87]. If we acknowledge the importance of interspecific interactions for limiting parasite growth, we should expect that each parasite is less virulent under coinfection than when alone even if coinfection selects for more virulent parasites.

It is also important to explore the impact of multiple infections on parasite population size. This is because infection processes can depend on the densities that parasites attain within hosts (see **Outstanding Questions**). For example, infection success in coinfection with the eye flukes *Diplostomum pseudospathaceum* and *D. gasterostei* depend on the genetic identity of each player and their dose [72]. Further, negative effects on growth of gregarines *(Ascogregarina culicis*) and microsporidia *(Vavraia culicis)* in coinfection of mosquitoes were more prevalent when the competitor was at a higher dose [88], and high nematode doses reduced viral and nematode loads in coinfections of tree frogs [89]. Conversely, changes in response to interspecific competitors were only observed at low intraspecific densities in transmission related-traits in the spider mite *T. urticae* in coinfection with *T. evansi* [90], and for *Plasmodium* load in coinfections with a helminth species in mice [91]. These results highlight the fact that changes in population size can have far-reaching effects on whether coinfections occur and their consequences.

In sum, our review shows that the field of host-parasite interactions would benefit from accounting for the specificities of interspecific interactions among coinfecting parasites. We hope this will contribute to more fluent communication between the fields of host-parasite interactions and community ecology as previously advocated [92]. Such integration will pave the road to consider a rich diversity of interspecific interactions among parasites, such as intraguild predation, apparent competition, or keystone predation [93] the consequences of which to the outcome of infections is still poorly understood.

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Declaration of interests

The authors declare no competing interests.

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Glossary

Coinfection: two or more different strains or species of parasite infecting the same individual host.

Cotransmission: simultaneous transmission of more than one parasite.

Multiple infection: two or more strains or species of parasite circulating in the same host population. This includes coinfections and super-infections.

Priority effect: scenarios where the outcomes of species interactions depend on their relative arrival times or initial abundances [94].

Reproductive interference: interspecific sexual interaction which reduces the fitness of at least one of the interacting species.

Stabilising effect: a mechanism that limits species dominance when abundant, but also protects them from extinction. It occurs for instance when intraspecific competition is stronger than interspecific competition.

Super-infection: One parasite genotype always displaces the other in a coinfected host.

Virulence: parasite-induced decrease in host fitness.

Box 1. Interspecific infections and the evolution of virulence

Generally, it is expected that multiple infections select for higher virulence. This is because competition for shared resources within a host is associated with selection for greater exploitation, hence, virulence [4]. However, higher virulence under coinfections is not always the predicted outcome (see **Table 1**). Relatedness among coinfecting parasites can decrease virulence, as prolonged host survival is expected to lead to increased fitness of the parasite offspring, but also that of their kin [95,96]. Lower virulence is also predicted when unrelated parasites are exposed to local competition (soft selection) [97]. Moreover, direct (interference) competition among parasites can also be associated with reduced virulence if the production of toxins is costly, if relatedness is intermediate [41], or when interactions among parasites are not symmetric (e.g. impact of parasite A on parasite B $\neq$ impact of parasite B on parasite A) [98]. Finally, facilitation among parasite species may alleviate the effect of within-host competition and/or strategies permitting efficient within-host exploitation and thus also favour reduced virulence [13].

Whether these outcomes are more relevant for intraspecific or interspecific parasite interactions is still poorly understood because the relative importance and

frequency of each is not known, even for individual parasite species (but see [17,19]). We can hypothesise (based on coexistence theory, see **Box 2**) that if interspecific competition were systematically stronger than intraspecific competition, interspecific coinfections would not be observed. Further, certain types of interactions may be more relevant for individuals of the same or different species. For instance, theory predicts that the evolution of virulence can depend on how parasites interact with each other via the immune system (e.g., [42,87,99]). Such predictions, generated by shared immune responses, should apply more to intraspecific interactions (or to closely-related species), as the probability that coinfecting parasites generate a similar immune response is higher than for more taxonomically-distant parasites (but see [100]). Interspecific parasite interactions mediated via the immune system may be more likely when there is negative immune cross-talk among different branches of the immune system [7,84].

Finally, if parasite responses to sharing their host with another species or strain are plastic, such that they adopt different strategies under co- *vs.* single infections, coinfection may have no effect on virulence evolution [87] [101].

Box 2. Coexistence theory and parasite infection outcomes in coinfections.

Coexistence theory posits that the ratio between the strength of interspecific and intraspecific interactions defines the magnitude of the stabilising effects [102]. The prediction is that the greater the stabilising effect (the more parasites limit their own growth relative to that of their competitor) the greater the chances for coexistence. These stabilising effects, arising under negative frequency dependence, need to be compared with the magnitude of equalising effects (the parasite´s intrinsic growth rate weighted by their overall sensitivity to competition) [103,104]. Conversely, parasites can limit the growth of their competitors more than that of themselves. Under such positive frequency dependence priority effects will exclude (or maintain at low abundance) species arriving later in the community (see **Figure I**).

Figure I. A coexistence theory map. This map shows different competition outcomes for multiple infections, based on the relationship between the stabilising effect of niche

693 differences (promoting parasite coexistence) and fitness differences (promoting
694 dominance of a single parasite; see [104]). Niche differences ($1-\rho$, being $\rho=\sqrt{\frac{\alpha_{ij}\,\alpha_{ji}}{\alpha_{ii}\;\alpha_{jj}}}$)
695 are estimated as a geometric average of the interspecific interactions ($\alpha_{ij}\;\alpha_{ji}$) divided by
696 the intraspecific interactions ($\alpha_{ii}\;\alpha_{jj}$) (the α´s measure the per-capita effect that one
697 individual of species *j* or *i* has, on average, on reducing copies of another strain or
698 species *i* or *j*, respectively). Positive niche differences correspond to competing
699 parasites engaging into negative density-dependent processes leading to within-host
700 coexistence of the two parasites (coinfection) or exclusion of the inferior competitor
701 (superinfection). Negative niche differences means competing parasites engage in
702 positive density-dependent processes resulting in superinfections or priority effects
703 (priorinfections, sensu [9]). Under priorinfections, the first parasite arriving in a host
704 precludes infection by the second, but both parasites can persist across a host
705 population if parasites infect different host individuals first. Fitness differences
706 ($\frac{\kappa_j}{\kappa_i}$, logarithmic scale) indicate the ability of a parasite to outcompete others. Fitness
707 differences are measured as the average ability of a parasite to produce new copies
708 $\left(\frac{\lambda_j-1}{\lambda_i-1}\right)$ weighted by their sensitivity to intra and interspecific competition $\left(\sqrt{\frac{\alpha_{ij}\,\alpha_{ii}}{\alpha_{jj}\;\alpha_{ji}}}\right)$ (see
709 [104] for details). Names of regions on the map state definitions from coexistence theory
710 and corresponding terms from the field of multiple infections [9]. With this map, it is
711 possible to study how different interaction mechanisms can change the outcome of
712 multiple infections. Here, we provide a hypothetical example (shown by the dots
713 changing from red to dark green with the arrow) in which the prediction of superinfection
714 changes to coinfection by reducing fitness differences and by increasing niche
715 differences. This might occur when competing parasites evolve to infect different host
716 compartments.
717

718 **Table 1. Consequence of different interactions among parasites in multiple infections for virulence.**

Type of interaction	Theoretical prediction for virulence evolution	Experimental examples consistent with theory for different parasite species	Experimental examples consistent with theory for different parasite strains
Competition			
Resource mediated competition	↑ Competition for shared host resources selects for more rapid growth [87,95].	Coinfection with hookworms (*Necator americanus* & *Ancyclostoma lumbricoides*) reduce malaria *(Plasmodium vivax*) loads in mice probably due to competition for red blood cells [83]	**A virulent malaria (*P. chabaudi*) strain has higher growth in mice and higher transmission to mosquitoes in coinfection [5]**
Apparent competition mediated via host immune system	↑ Competition mediated via the immune system can select for more rapid growth to escape the immune response [42,99]	One bacterium (*Haemophilus influenzae*) outcompetes another (*Streptococcus pneumoniae)* by inducing the host immune response (virulence or parasite fitness was not reported) [12]	An avirulent malaria (*P. chabaudi*) strain has higher densities in immunodeficient mice [105]

Competition for public goods	↓↑ Competition selects against public good production and therefore lower growth [96,106] Coinfection with public goods can lead to higher virulence, depending on the shape of the trade-off [107]	**Evolution leads to reduced virulence and public good production in a pathogenic bacterium (*Staphylococcus aureus*) coinfecting nematode hosts with a protective bacterium (*Enterococcus faecalis*) [108]**	Infections with a public good producing bacterial strain (*Pseudomonas aeruginosa*) have higher growth and are more virulent in their insect host than coinfections with a cheater strain that does not invest in public goods [109]
Competition mediated via toxins, spite	↓ Competition mediated via the production of toxins is costly and thus selects for lower growth [41] Spite selects for reduced virulence, except when cost is low and the interaction symmetric [98]	Interference competition among different parasitic nematode species via their bacterial symbionts (*Xenorhabdus* spp.) is associated with lower virulence (*i.e*, lower mortality of the nematode insect host) [41] Two insect-killing bacteria species (*Xenorhabdus sp.* and *Photorhabdus sp.*) that produce bacteriocins have decreased	Evolution under mixed *Bacillus thuringiensis* bacteria infections in insect hosts results in lower parasite growth, hence lower virulence, because bacteria invest in toxins that kill competitors rather than in their own growth,a pattern consistent with spite [111]. Note, however, that these toxins may also be

		virulence under coinfection [110]	considered a public good.
Superinfection	↓↑ Superinfection selects for more virulent parasites that replace less virulent ones in a host. It can also lead to evolutionary branching with a less virulent strain evolving to infect new hosts sooner [8]	Superinfection among closely related prophages in murine bacterial host prevents infection with other prophages and extrachromosomal viruses (virulence was not measured) [112].	Higher plasmid virulence correlates with superinfection ability of *Escherichia coli* bacterial hosts [113]
Facilitation			
Immunosuppression or immune trade-off	↓ ↑ Immunosuppression can select for lower virulence if it increases parasite load and host mortality [98] Immune impairment can select for	Immunosuppression by helminths in coinfection with bovine tuberculosis reduces mortality induced by the latter in African buffalo hosts [114]	

	lower virulence in coinfection in the absence of competition between genotypes. Higher virulence is selected under competition [87] Immunosuppression may increase the prevalence of coinfected hosts in the population, and thus selection for higher virulence [32]		
Cotransmission	↓ ↑ Cotransmission can select for reduced virulence as the interests of the two strains are aligned, but it depends on the initial virulence of each coinfecting parasite [14]	Increased fecundity of spider mite hosts coinfected with vertically transmitted *Wolbachia* & *Cardinium* bacteria [115]. Arthropods that vector plant viruses often have higher fitness when feeding on virus infected plants due to negative immune cross talk, or the release of free amino acids in the plant [26]	High relatedness among malaria (*P. falciparum*) strains in coinfection suggests co-transmission rather than independent acquisition of lineages which may reduce levels of within-host competition and selection for higher virulence [116].

719 In the 'Theoretical prediction for virulence evolution' column, upper (↑) and lower (↓) arrows indicate higher and lower
720 virulence, respectively, whereas the equal sign (=) indicates no overall change. The table shows the type of interaction

among parasites and the theoretical prediction for virulence evolution. Where possible, experimental examples of the interaction mechanism are provided for both inter- and intraspecific multiple infections, alongside the consequence for virulence and/or parasite fitness. Highlighted in bold are experimental studies that are congruent with theoretical predictions regarding the interaction mechanism and the outcome for virulence evolution. Note that not all studies are the result of experimental evolution or use genetically distinct lineages, hence changes in virulence in response to the presence of competitors may be due to plasticity rather than evolution. Predictions for reproductive interference and virulence evolution are not included due to a lack of studies.