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- 1 Interspecific interactions among parasites in multiple infections
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- 20

21 Abstract

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

32

33 The diversity of multiple infections

34 Parasites are ubiguitous and host populations are often infected with more than one 35 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 36 37 parasite life-history and evolution. They can affect their virulence, as well as other traits 38 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 39 different strains of *Plasmodium chabaudi* in mice [5]) or to different species, which can 40 41 be closely related (e.g. ectoparasitic mites *Tetranychus urticae* and *T. evansi* on tomato 42 plants [6]) or from highly-differentiated guilds (e.g., *Plasmodium* or other microparasites 43 and helminth worms in mice [7]). When parasites co-occur in a host population, 44 interactions in the within-host environment are mostly described by super-infections or 45 co-infections [8], although other possibilities exist [9]. Within hosts, the most common 46 interaction among parasites may be competition for resources [10]. However, they can 47 also engage in interference competition via the production of toxins [11], competition 48 mediated by the immune system [12]; or they may instead facilitate each other (e.g. 49 [13]). In addition, they can be co-transmitted among hosts with variable consequences 50 for parasite evolution [14] (see Table 1). 51 This review aims to show the importance of being explicit about whether parasites in 52 multiple infections belong to the same or a different species. We thus explore the scope

53 for interspecific interactions among parasites in multiple infections to have specific

54 impacts on hosts and parasites. First, we address factors that may promote the

55 occurrence of interspecific multiple infections in host populations. We then address

56 where interspecific interactions fit into the theory of the evolution of virulence. Next, we

57 report how interspecific competition can be coupled with intraspecific competition to

58 predict the outcome of coinfections within hosts. We subsequently discuss how genome

59 integration among different species and their sexual interactions may affect host-

60 parasite interactions. Finally, we address the role of interspecific interactions in

61 maintaining host and parasite diversity.

62

63 Factors promoting the prevalence of multiple interspecific infections in host populations: co-occurrence, within-host interactions and co-transmission 64 65 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 66 that parasites from different species co-occur in time and space and encounter one 67 68 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 69 shared life-history traits, this may not be the case for parasites of different species. Still, 70 71 many factors may promote the co-occurrence of multiple parasite species in a host 72 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].

80 Non-random associations among parasites in multiple infections may result from 81 within- or between-host interactions. Within the host, increased virulence due to 82 coinfection may result in fewer coinfections than expected by single species/strain 83 distributions [20]. Whether this is more or less likely under inter- or intraspecific 84 interactions will hinge upon how these interactions affect parasite fitness (see **Box 1**). Within-host interactions also affect species composition in coinfections. For instance, 85 86 the tomato infecting bacterium Pantoea dispersa is more resistant to invasion by 87 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* 88 89 with different *Podosphaera plantaginis* bacterial strains is more likely when their fitness 90 is similar [22] and when particular bacterial species infect first [23]. Whether such 91 interactions favour the co-occurrence of different parasite species will hinge upon their 92 relative effect on parasites from the same or different species (see section 3).

93 Parasite interactions during between-host transmission may also affect the prevalence of coinfections. Indeed, parasite species are more likely to co-occur within 94 95 the same individual host if they are **cotransmitted**. Some parasites of different species 96 are obligately co-transmitted. For example, satellite nucleic acids are encapsidated by 97 capsid proteins of their helper virus [24]. Also, several parasites are vectored by other 98 pest/parasite species, as is the case of tomato spotted wilt virus, which relies on thrips 99 for transmission. Thrips are important crop pests and co-transmission has been shown 100 to be beneficial to both the virus and its vector, as the virus benefits by being 101 transmitted, whereas thrips have higher fitness on virus-infected plants [25]. This 102 pattern is true for many virus-vector systems [26]. In both these cases only one parasite 103 relies on another for transmission. However, we are not aware of any examples where 104 two horizontally transmitted parasites are both mandatorily cotransmitted.

105 Cotransmission may also emerge because parasites share the same 106 transmission mode or route, such as certain sexually transmitted chronic diseases [27] 107 or vertically transmitted symbionts [28]. Parasites that share the same vector species 108 may also be cotransmitted, such as the arboviruses dengue, chikungunya and Zika, 109 vectored by the mosquito Aedes aegypti [29] or plant viruses vectored by the whitefly 110 Bemisia tabaci [30]. However, potential competitive interactions within the vector may 111 hinder the successful transmission of all parasites to the next host [30]. Increased 112 coinfection prevalence may also arise from transmission by the same vector but at 113 different transmission events, especially if the prevalence of viruses and vectors is high 114 [31].

115 Even if parasite transmission is independent, coinfections comprising different 116 parasite species may be more likely to occur if parasites are more attracted, or 117 infectious, to hosts harbouring another species of parasite. Immunosuppression of the 118 host is one mechanism that might lead to increased coinfection [32]. In line with this, 119 Gammarus pulex infected with the acanthocephalan Pomphorhynchus laevis are more 120 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 121 122 wilt virus, possibly due to negative immune cross-talk preventing the host from mounting 123 an effective immune response against spider mites [34].

124

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

127 The study of interactions among parasites within hosts has focused more on the 128 outcomes of infections rather than on the underlying processes governing such 129 outcomes [4,35]. This black-box approach has led theory to focus on either super-130 infections (e.g., [36]) or coinfections (e.g., [37]). These approaches do not account for 131 the plethora of possible interactions among parasites, which has led to recent work 132 claiming the need for a more mechanistic approach to the study of coinfections [9,38]. 133 Indeed, it is only by understanding the mechanisms underlying parasite interactions that 134 it may be possible to predict outcomes and potential parasite evolution in coinfections 135 [39,40].

136 Coexistence theory (**Box 2**) emerges as a natural foundation to address 137 coinfections mechanistically for several reasons. First, it accommodates different 138 interactions among parasites, from apparent to interference competition [41,42] to 139 facilitation [13]. Second, its recent extensions to network theory [43] allow incorporating 140 interactions between parasites and hosts, which is key to establishing a solid link 141 between multiple infections and the evolution of virulence. Finally, although coexistence 142 theory is a general framework for community ecology, it predicts the exact same variety 143 of outcomes as those predicted by frameworks specifically based on multiple infections 144 ([9]; see **Box 2**).

145 A few highly controlled experiments have used coexistence theory in systems with 146 parasites [6,44,45]. For instance, Fragata et al, (2022) [6] show that *T. evansi* generally 147 excludes T. urticae except when the latter arrives first to a host plant and occupies T. 148 evansi's preferred niche, in which case the two species are predicted to coexist in a 149 coinfection scenario. This work highlights the importance and relevance of combining 150 the fields of Host-Parasite Interactions with that of Coexistence Theory, by showing that 151 the concepts of competitive exclusion and priority effects are relevant to multiple 152 infections (Box 2). Yet, many potential avenues for future research remain to be 153 uncovered. In particular, we lack understanding of how multiple infection outcomes, as 154 predicted by coexistence theory, connect with emerging infection properties such as

virulence and parasite fitness (R₀, the number of new hosts that become infected from a
given infection in a susceptible population for microparasites or the number of infectious
stages produced per infection period for macroparasites, [46]). For instance, we could
hypothesise that virulence and stabilising effects might be negatively related because
the stronger the stabilising effect, the more parasites specialise on different resources
(niches), leading to fewer negative interactions among them, reducing selection for
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].

187 Reproductive interference and competition may also affect the production of 188 sexual stages in haemosporidian parasites such as *Plasmodium* [54,55]. Reproductive 189 interference between parasite species can hamper sexual reproduction, which occurs in 190 the vector for this parasite species [54,55]. This could select for higher asexual 191 replication within the vertebrate host. Alternatively, interspecific competition may directly select for asexual growth in the vertebrate host, as observed in mixed strain infections 192 193 [58]. Hence, both types of interaction (within-host competition and reproductive 194 interference) may drive increased virulence but reduced or delayed transmission and 195 possibly asynchrony in the production of sexual stages and sexual reproduction among 196 parasite species (e.g. [55]).

197

198 Across species incorporation of genetic material

199 Some empirical studies have reported that parasites may incorporate DNA from other 200 species, changing life-history traits and impacting epidemics. This can occur via 201 hybridisation between closely related parasite species (reviewed in [59]) or the transfer 202 of genetic material among more distant species. Indeed, hybrid parasites may have 203 higher virulence (e.g. *Trypanosoma* [60]), transmission potential (e.g. *Leishmania*, [61]) 204 and a greater host range than their parental species (e.g. Schistosoma [62]). Gene 205 transfer of the virulence gene ToxA from the wheat pathogen Stagonospora nodorum 206 into the non-pathogenic fungus Pyrenophora tritici-repentis leads to the production of 207 pathogenicity-related toxins in the latter [63]. Horizontal gene transfer among plant 208 species may have increased the adaptive potential of some Orobranchaceae parasitic 209 plants to their hosts [64]. In addition, viruses with segmented genomes may exchange 210 entire segments, a process called reassortment, even among distantly related 211 genotypes (e.g. with > 50% nucleotide divergence [65]). Reassortment among avian 212 influenza lineages with lineages circulating in human populations have been responsible 213 for the past three human epidemics [66]. These events of genetic exchange, although 214 probably stochastic and/or rare, are noted since they confer a parasite with a fitness 215 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

- 217 among more distant parasite species may be less prevalent, hence harder to foresee or
- 218 identify after they occur. In any case, there is a large scope for genetic exchange during
- 219 coinfections with interspecific parasites which can in turn affect important life-history
- traits, including virulence [59,63,67].
- 221

222 Impact of interspecific coinfections on host and parasite diversity

223 Infections with parasites from different species, by exposing hosts to a more variable 224 environment, hold the potential to maintain genetic diversity in both parasite [68,69] and 225 host [70,71] populations, via G_PxG_PxG_H interactions. There is indeed evidence that 226 parasitic traits may vary with the identity of the infecting species. For example, infection 227 success of the fish eye flukes *Diplostomum pseudopathaceum* and *D. gasterostei* 228 varies with the combination of the competing strains, increasing or decreasing by up to 229 \sim 30% depending on initial dose [72]. Also, the outcome of competition between 230 nematodes depends on the genotype of different species of Xenorhabdus bacterial 231 symbionts and whether interactions are mediated via toxins (reduced growth and insect 232 host mortality) [11]. Similarly, different population combinations of the ectoparasites 233 Tetranychus urticae and T. evansi lead to higher or lower numbers of each species 234 under coinfection vs. single infection [73]. Importantly, the traits observed in these 235 multiple infections are not necessarily predictable from observations in the 236 corresponding single infections [11,72,73]. Therefore, coinfections lead to variation in 237 infection-related traits, which in turn may result in different selection pressures that 238 hosts and parasites are exposed to, potentially maintaining variation in their 239 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

- 247 exposure of bacterial hosts (*Pseudomonas aeruginosa*) to more variable virus
- 248 communities has no effect on within-population genetic diversity and leads to increased
- 249 divergence among populations [78].
- 250

251 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.

259 Arguably the most relevant knowledge gap is the prevalence of interspecific coinfections in natural populations. Currently, available information on this issue is 260 261 scattered and mostly concentrated in a few model systems (e.g. [16-18,79]). We lack 262 ecological knowledge on the relative proportion of coinfections by different parasite 263 species compared to coinfections by conspecific parasites, and single infections (see 264 **Outstanding Questions**). This knowledge is key to unravelling the forces shaping epidemiology and evolutionary processes in both hosts and parasites. Advances in 265 266 genomic and metabarcoding approaches that will enable simultaneous identification of 267 multiple parasite species may shed light on this.

268 Next, it is also key to identify the factors governing the prevalence of 269 coinfections. One hypothesis drawn from coexistence theory is that the likelihood of 270 coinfection depends on the phylogenetic distance among competing parasites [80]. If 271 more distantly related parasites have diverged in their resource requirements and have 272 resulted in reproductive barriers, then such stabilising niche differences may promote 273 their coinfection. Conversely, if genetic changes have increased the competitive ability 274 of one parasite over the other, then superinfection is expected, and coinfections would 275 only occur among close relatives. Both outcomes have been documented in the 276 literature on plant community ecology [81,82], but this information is largely missing for 277 multiple infections. Using community ecology principles may be instrumental for a

278 deeper understanding of multiple infections. This was illustrated by Budischak et al 279 (2018) [83] and Graham et al (2008) [7], who showed that outcomes of coinfection can 280 be predicted based on whether interactions are bottom-up (competition for shared host 281 resources) or top-down (mediated via host immune responses). Moreover, depending 282 on the phylogenetic distance, interacting mechanisms among parasites may differ (see 283 **Outstanding Questions**). For instance, closely-related parasites may interact more via 284 competition [6] and/or reproductive interference [52] within the host, while an interaction 285 more characteristic of distantly-related species may be via negative cross-talk between 286 different branches of the host immune system (e.g., [7,84]). It should be noted that a 287 recent meta-analysis across 424 species pairs (269 species) in free-living organisms 288 found that functional similarity, rather than phylogenetic relatedness, predicted the 289 strength of interspecific competition and thus may better explain patterns of community 290 assembly [85]. Whether this is also true for parasites in coinfections remains to be 291 tested (but see [7]). Such studies further understanding about general rules that can be 292 applied to predict the outcomes of coinfections comprising different parasite species.

293 The phylogenetic distance or functional similarity between coinfecting parasites 294 may also affect virulence (see **Outstanding Questions**). To address this issue, detailed 295 comparisons between coinfections with conspecifics and heterospecifics are needed. 296 Such a comparison has been made by Staves and Knell (2010) [86], who documented 297 higher virulence on a wax moth when coinfected with different strains of the fungus 298 Metarhizium anisopliae, but lower virulence when the coinfection included an 299 entomopathogenic nematode [86]. More such examples are needed. Another related 300 knowledge gap is the relative contribution of each parasite to the overall virulence of 301 coinfections. Measuring this may be more feasible when parasites are from different 302 species if symptoms are distinct and can be linked to the fitness of each parasite. This 303 information is important to understand when virulence under coinfection is higher or 304 lower compared to the sum of the virulence of each parasite separately [87]. If we 305 acknowledge the importance of interspecific interactions for limiting parasite growth, we 306 should expect that each parasite is less virulent under coinfection than when alone even 307 if coinfection selects for more virulent parasites.

308 It is also important to explore the impact of multiple infections on parasite 309 population size. This is because infection processes can depend on the densities that 310 parasites attain within hosts (see **Outstanding Questions**). For example, infection 311 success in coinfection with the eye flukes *Diplostomum pseudospathaceum* and *D*. 312 gasterostei depend on the genetic identity of each player and their dose [72]. Further, 313 negative effects on growth of gregarines (Ascogregarina culicis) and microsporidia 314 (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 315 316 in coinfections of tree frogs [89]. Conversely, changes in response to interspecific 317 competitors were only observed at low intraspecific densities in transmission related-318 traits in the spider mite T. urticae in coinfection with T. evansi [90], and for Plasmodium 319 load in coinfections with a helminth species in mice [91]. These results highlight the fact 320 that changes in population size can have far-reaching effects on whether coinfections 321 occur and their consequences.

322 In sum, our review shows that the field of host-parasite interactions would benefit from 323 accounting for the specificities of interspecific interactions among coinfecting parasites. 324 We hope this will contribute to more fluent communication between the fields of host-325 parasite interactions and community ecology as previously advocated [92]. Such 326 integration will pave the road to consider a rich diversity of interspecific interactions 327 among parasites, such as intraguild predation, apparent competition, or keystone 328 predation [93] the consequences of which to the outcome of infections is still poorly 329 understood.

330

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- 628 Glossary

629 **Coinfection**: two or more different strains or species of parasite infecting the same

630 individual host.

- 631 **Cotransmission**: simultaneous transmission of more than one parasite.
- 632 **Multiple infection:** two or more strains or species of parasite circulating in the same
- 633 host population. This includes coinfections and super-infections.
- 634 **Priority effect**: scenarios where the outcomes of species interactions depend on their
- relative arrival times or initial abundances [94].
- 636 **Reproductive interference**: interspecific sexual interaction which reduces the fitness of
- 637 at least one of the interacting species.
- 638 **Stabilising effect**: a mechanism that limits species dominance when abundant, but
- also protects them from extinction. It occurs for instance when intraspecific competition
- 640 is stronger than interspecific competition.
- 641 Super-infection: One parasite genotype always displaces the other in a coinfected642 host.
- 643 **Virulence**: parasite-induced decrease in host fitness.
- 644

645 Box 1. Interspecific infections and the evolution of virulence

646 Generally, it is expected that multiple infections select for higher virulence. This is 647 because competition for shared resources within a host is associated with selection for 648 greater exploitation, hence, virulence [4]. However, higher virulence under coinfections is not always the predicted outcome (see Table 1). Relatedness among coinfecting 649 650 parasites can decrease virulence, as prolonged host survival is expected to lead to 651 increased fitness of the parasite offspring, but also that of their kin [95,96]. Lower 652 virulence is also predicted when unrelated parasites are exposed to local competition 653 (soft selection) [97]. Moreover, direct (interference) competition among parasites can 654 also be associated with reduced virulence if the production of toxins is costly, if 655 relatedness is intermediate [41], or when interactions among parasites are not 656 symmetric (e.g. impact of parasite A on parasite $B \neq$ impact of parasite B on parasite A) 657 [98]. Finally, facilitation among parasite species may alleviate the effect of within-host 658 competition and/or strategies permitting efficient within-host exploitation and thus also 659 favour reduced virulence [13].

660 Whether these outcomes are more relevant for intraspecific or interspecific 661 parasite interactions is still poorly understood because the relative importance and 662 frequency of each is not known, even for individual parasite species (but see [17,19]). 663 We can hypothesise (based on coexistence theory, see **Box 2**) that if interspecific 664 competition were systematically stronger than intraspecific competition, interspecific 665 coinfections would not be observed. Further, certain types of interactions may be more 666 relevant for individuals of the same or different species. For instance, theory predicts 667 that the evolution of virulence can depend on how parasites interact with each other via 668 the immune system (e.g., [42,87,99]). Such predictions, generated by shared immune 669 responses, should apply more to intraspecific interactions (or to closely-related 670 species), as the probability that coinfecting parasites generate a similar immune 671 response is higher than for more taxonomically-distant parasites (but see [100]). 672 Interspecific parasite interactions mediated via the immune system may be more likely 673 when there is negative immune cross-talk among different branches of the immune 674 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].

678

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

680 Coexistence theory posits that the ratio between the strength of interspecific and 681 intraspecific interactions defines the magnitude of the stabilising effects [102]. The 682 prediction is that the greater the stabilising effect (the more parasites limit their own 683 growth relative to that of their competitor) the greater the chances for coexistence. 684 These stabilising effects, arising under negative frequency dependence, need to be 685 compared with the magnitude of equalising effects (the parasite's intrinsic growth rate 686 weighted by their overall sensitivity to competition) [103,104]. Conversely, parasites can 687 limit the growth of their competitors more than that of themselves. Under such positive 688 frequency dependence priority effects will exclude (or maintain at low abundance) 689 species arriving later in the community (see Figure I).

690

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

693 differences (promoting parasite coexistence) and fitness differences (promoting dominance of a single parasite; see [104]). Niche differences $(1 - \rho, \text{being } \rho = \sqrt{\frac{\alpha_{ij} \alpha_{ji}}{\alpha_{ii} \alpha_{ij}}})$ 694 are estimated as a geometric average of the interspecific interactions ($\alpha_{ii} \alpha_{ii}$) divided by 695 696 the intraspecific interactions ($\alpha_{ii} \alpha_{ij}$) (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 population if parasites infect different host individuals first. Fitness differences 705 $\binom{\kappa_j}{\kappa_j}$ logarithmic scale) indicate the ability of a parasite to outcompete others. Fitness 706 differences are measured as the average ability of a parasite to produce new copies 707 $\left(\frac{\lambda_{j}-1}{\lambda_{i}-1}\right)$ weighted by their sensitivity to intra and interspecific competition $\left(\sqrt{\frac{\alpha_{ij} \alpha_{ii}}{\alpha_{ij} \alpha_{ij}}}\right)$ (see 708 709 [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

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 (<i>Necator americanus</i> & <i>Ancyclostoma lumbricoides</i>) reduce malaria (<i>Plasmodium</i> <i>vivax</i>) loads in mice probably due to competition for red blood cells [83]	A virulent malaria (<i>P. chabaudi</i>) 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 (<i>Haemophilus</i> <i>influenzae</i>) outcompetes another (<i>Streptococcus pneumoniae</i>) by inducing the host immune response (virulence or parasite fitness was not reported) [12]	An avirulent malaria (<i>P. chabaudi</i>) strain has higher densities in immunodeficient mice [105]

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

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 (<i>Staphylococcus</i> <i>aureus</i>) coinfecting nematode hosts with a protective bacterium (<i>Enterococcus</i> <i>faecalis</i>) [108]	Infections with a public good producing bacterial strain (<i>Pseudomonas aeruginosa</i>) 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 (<i>Xenorhabdus</i> spp.) is associated with lower virulence (<i>i.e</i> , lower mortality of the nematode insect host) [41] Two insect-killing bacteria species (<i>Xenorhabdus sp.</i> and <i>Photorhabdus sp.</i>) 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

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]	virulence under coinfection [110] Superinfection among closely related prophages in murine bacterial host prevents infection with other prophages and extrachromosomal viruses (virulence was not measured) [112].	considered a public good. Higher plasmid virulence correlates with superinfection ability of <i>Escherichia coli</i> 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]	

719 In the 'Theoretical prediction for virulence evolution' column, upper (\uparrow) and lower (\downarrow) 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.