

## Importance of sequence and timing in parasite coinfections

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### Abstract

Coinfections by multiple parasites predominate in the wild. Interactions between parasites can be antagonistic, neutral or facilitative, and they can have significant implications for epidemiology, disease dynamics and evolution of virulence. Coinfections commonly result from sequential exposure of hosts to different parasites. We argue that the sequential nature of coinfections is important for the consequences of infection in both natural and manmade environments. Coinfections accumulate during host lifespan, determining the structure of the parasite infracommunity. Interactions within the parasite community and their joint effect on the host individual potentially shape evolution of parasite life-history traits and transmission biology. Overall, sequential coinfections have the potential to change evolutionary and epidemiological outcomes of host-parasite interactions widely across plant and animal systems.

## Temporal Sequence in Parasite Coinfections

During its life span, the same host individual encounters a multitude of different parasites. Indeed, **coinfection** (see Glossary) by multiple parasite species or strains/genotypes is proving to be the rule in both natural and manmade environments [1, 2] (we use the term “coinfection” to include scenarios with and without parasite coexistence (Box 1) unless noted otherwise). To date, the majority of research on coinfections assumes simultaneous arrival (or ignores arrival order) although in reality in most systems we expect different parasites to arrive sequentially. The arrival sequence of different parasites may be predictable for example due to phenology/seasonality, or in many cases unpredictable because of haphazard contacts between hosts and infective propagules. In this opinion article, we argue that the arrival sequence of different parasites is an important determinant of parasite infection success and virulence in the wild. We review recent literature showing that a prior residency in a host can alter coinfection success across diverse host taxa such as plants [3-5], invertebrates [6, 7] and vertebrates [8-11] (Fig. 1).

Interactions among parasites sharing the same host can be antagonistic, facilitative or neutral, but, in principle, coinfecting parasites can be considered as competitors that have conflicting interests in use of host resources for growth and reproduction [12, 13], to secure **transmission** to the next host [14, 15], and even how host should behave in order to facilitate transmission [16]. Antagonistic interactions can take place directly in the form of competitive interference or exclusion between coinfecting parasites [17-19], or indirectly through resource competition or apparent competition mediated by cross-reactive host immune responses [20, 21]. Facilitation, on the other hand, could follow from one parasite suppressing the immune function of the host [12] or from coinfection representing an additional challenge to the host immune system ([22, 23], review in [24]). In theory, coinfection interactions should also be stronger between closely related parasites (strain or genotypes of one parasite species, or closely related parasite species) because of similarities in the transmission process, elicited immune recognition profiles, apparent and realized competition between the coinfecting partners etc. [25, 26]. However, recent evidence also supports interactions between unrelated coinfecting parasites, at least in some taxa [27-31]. Overall, coinfections may change the **virulence** the host experiences and interactions among parasites can directly affect their **fitness**. Therefore, coinfections can have significant implications for epidemiology, disease dynamics and evolution of virulence (reviews e.g. in [32-34]).

Coinfections, like many other features of host-parasite interactions, are temporally dynamic. This means that two parasites are more likely to infect the host sequentially rather than simultaneously. The time gap between infections can vary from few moments to a significant proportion of the host lifespan, where the longer-term effects require the first infection to become chronic or to elicit a long-lasting host response. Further, the type of host exposure, simultaneous or sequential, depend on the specific details of the infection process and transmission of each of the parasites. For example, simultaneous infection of a host (Box 1) may be common when a disease vector such as a tick carries multiple viral and bacterial infections, and co-transmits these infections to the next host [35]. A more common co-transmission scenario may arise when coinfecting **intermediate hosts** of trophically transmitted parasites are consumed by the predatory next host. Further, infective stages that penetrate host epithelium could, in theory, open a route to host body for other infections or act as carriers of microbes (see [31] for such an interaction). However, we argue that such circumstances are much less common than situations where host exposure to different parasites varies through time (Box 1). Such variation is driven by the significant spatiotemporal heterogeneity associated with host-parasite interactions in the wild [36]. More specifically, spatial aggregation of infected hosts and that of the infective stages [37-39], and the temporal variation in parasite transmission biology [40, 41], result in a mosaic of hotter and colder spatiotemporal spots of infection, specific to each parasite [42]. Consequently, hosts become exposed to different parasite propagules at different times.

Here, we focus primarily on ecological literature, but acknowledge the wealth of medical literature on the topic considering pathogen interactions, effects of vaccines etc. in epidemiology of human diseases. We also mainly focus on the role of the host immune system in mediating sequential infections, although we acknowledge also other possible forms, such as direct interactions, between parasites. We first consider implications of sequential coinfections for virulence in the hosts, pointing out areas of research that have received less attention. Second, we consider how ecological and evolutionary consequences of simultaneous and sequential coinfection may differ for parasite epidemiology and transmission strategies. Overall, we propose that sequential processes of coinfections may influence many, if not most, host-parasite-parasite interactions in nature.

### **Sequential infections and implications for virulence**

Theoretically, coinfecting parasite species and individuals can interact to a degree that drives evolution of virulence (reviews in [1, 32]) and maintains fitness variation in parasite populations (review in [43]). Many of these predictions still await for comprehensive empirical support. Multiple parasite infections, simultaneous and sequential, have traditionally been approached through models of coinfection (here coexistence of two parasite strains of one species or two different species) and superinfection (total exclusion of one strain by other without coexistence), showing that order of arrival can significantly change the outcome of virulence [32]. However, as recently pointed out by Sofonea et al. [44], the complexity of multiple parasite infections is unlikely to be captured by the coinfection-superinfection dichotomy alone. For example, many coinfection models do not consider host recovery (or parasite clearance) [32], which is important if the first (sequential) infection elicits a long-lasting, cross-reactive immune response that prevails after clearance and influences subsequent parasites (Box 1). Second, infections can be chronic and prevail in hosts for years (e.g. helminths), which can influence outcomes of many other (acute) infections that emerge and pass rapidly in an epidemic manner [12]. Similarly, the order of sequential infection can be important with the outcome being different when parasite A infects before B, compared to B before A (Box 1). Third, these scenarios can be influenced by whether infections are local or systemic; i.e. not all parasites interact, but this depends, for example, on the resources extracted, site of infection and type of host immune responses. Consequently, interactions in sequential infections and in multiple infections in general are more likely between related parasite species. Finally, host demography is important; young hosts can provide fewer resources, but can also show weaker immune responses after birth compared to older individuals that have already been exposed (repeatedly) to the same or different parasites. We argue that incorporating such dynamics into models of (sequential) coinfections would make them more realistic, but, inevitably, also more complex.

Empirical examples of sequential coinfections in plants (e.g. [3-5, 45]) suggest that arrival sequence of pathogen strains may be a key determinant of infection outcomes. There are examples of later arriving strains having lower success of establishment and this is attributed to induced host resistance, as the first arriving pathogen triggers host defenses that are effective against later arriving pathogens ('cross-protection', Table 1). Thus, sequence of arrival can have strong effects for within host pathogen dynamics. Indeed, simultaneous infections are often significantly more damaging to hosts than sequential coinfections [4]. Similar to plants, examples from invertebrate and vertebrate hosts suggest that temporal sequence between two

parasites can influence the infection outcome, mostly by mechanisms of resource competition and/or apparent host immune-mediated competition (Fig. 1; Table 1). In many cases, these effects are asymmetric [7, 10] and depend on the species and transmission mode of the first infecting parasite. Overall, the current evidence strongly highlights negative effects for the later arriving parasite (Table 1).

There are currently three major gaps in knowledge regarding the effect of sequential infections on virulence. First, to draw conclusions on the evolution of virulence, studies should not only compare virulence in simultaneous and sequential coinfections, but also look into genotype ( $G \times G$ ) interactions between the coinfecting parasites. This is necessary to gain understanding on which virulence genotypes are favored by selection [26, 32]. Recent studies on simultaneous infections of two parasites have shown the complexity of such interactions (e.g. [31, 46]), but similar approaches are lacking in sequential infections. We argue that empirical tests addressing  $G \times G$  interactions and virulence in sequential coinfection framework are necessary to gain a comprehensive understanding of virulence evolution in different infection backgrounds of hosts. Second, mechanisms underlying effects of sequential infections are generally poorly known. In most cases, they likely involve both direct (e.g. interference competition) and indirect interactions (resource competition, host immune-mediated apparent competition), but their relative contribution is often unknown (Table 1). We argue that elucidating such mechanisms is important as they underlie evolution of virulence in many, if not most, systems [32]. These mechanisms are also likely influenced by within-host dynamics (e.g. local vs. systemic, acute vs. chronic infections), as well as taxonomic relatedness of the parasites. Finally, while research on coinfections, simultaneous and sequential, is heavily based on strains of single species or closely related parasites, we propose that interactions between completely unrelated parasites are probably more common than previously anticipated. Thus, we encourage more research towards community-level patterns and processes of sequential coinfections to elucidate the breadth of possible direct and indirect interactions.

### **Implications of sequential coinfections for parasite transmission strategies and epidemiology**

Coinfections can represent opportunities or challenges also for parasites. Coinfection scenarios are typically unpredictable for the coinfecting parasites in terms of background of the target host (species, resistance genotype) and the identity of the coinfecting partner (genetic interaction between the parasite individuals; see [31, 46] for examples of simultaneous

coinfections). Sequential infections add yet another component to the unpredictable “host environment” that newly arriving parasites must face. In general, an uninfected host is a first-come-first-served resource, where sequential host exposure can result in direct competitive interference/exclusion, or indirect resource or host immune-mediated competition that the second invader needs to deal with (Box 1). An interesting question is if evolution of parasite traits has been responding to probability of coinfection, simultaneous or sequential.

In theory, host heterogeneity (uninfected/infected) could result in selection (evolutionary branching) towards specialized parasite strains, ones targeting uninfected hosts and others those already infected [25, 47]. However, given the wide spectrum of possible interactions in a coinfecting parasite infracommunity and parasite within-host dynamics (see above), we argue that these interactions probably are orders of magnitude more diverse in nature. Sequential exposure of hosts to one or several parasites could nevertheless influence evolution of transmission strategies. For example, the first infection could result in suppression of host immune function, allowing higher replication for the second one [12]. A simultaneous coinfection could also result in higher infection success for parasites in immunologically naïve host compared to single infection if the infection diversity represented a higher challenge for the host immune system ([22], review in [24]). However, such benefits of co-exposure could disappear following sequential exposure and activation of the host immune system (Box 2). In theory, the latter scenario could select for co-transmission strategies aiming at naïve hosts with lower resistance and possibility for facilitation in coinfection success. This could be possible, for example, if parasites coincided the release of infective stages with the emergence of young, susceptible host cohorts. However, facilitation requires that there is little or no competition between parasites, which is why such interactions are likely to be system specific. Overall, more research on facilitative interactions in sequential coinfections and transmission is needed in different systems.

Sequential host exposure to multiple parasites can have important implications also for parasite epidemiology. For example, a significant proportion of the host population being already infected or showing cross-reactive immune responses from previous infections can alter the success of the later arriving parasites (Box 1), thus potentially changing the course of an epidemic [48]. This is the fundamental element, for example, in vaccination programs, where a sequential administration of attenuated pathogens of one type can prevent epidemics of virulent strains through cross-reactive immune responses. However, most of the evidence on

sequential coinfections outside the medical realm comes from laboratory experiments (Table 1), where conditions often do not correspond to nature in terms of infection dose (unnaturally high doses) or pattern of exposure (order and administration of infection is forced). Thus, exploring the actual epidemiological consequences of sequential exposure requires approaches in the field. Recent investigations manipulating the order (sequence, priority effects) of infections have demonstrated significant changes in the epidemiology [45] and community structure [49] of parasites in natural conditions. For example, Halliday et al. [45] elegantly took advantage of natural sequence of multiple infections driven by environmental conditions and showed how the sequence and interactions between parasites influenced the epidemics.

We propose that many more experiments in natural conditions are needed to understand the general epidemiological consequences of sequentially occurring infections. It would be important also from an evolutionary perspective to implement ecological conditions of parasite coinfections that resemble better the natural patterns of parasite exposure and resulting interactions. Epidemiological implications of sequential infections have relevance also for prevention of diseases of humans and livestock. For instance, if previous or existing other infections could modulate the outcome of a target infection this could represent a challenge for effective disease mitigation strategies [12, 50]. This is well illustrated in intensive production units, where epidemics are commonly treated with little consideration of presence or history of other infections. In general, we argue that coinfections, simultaneous or sequential, can contribute to disease-related management failures and are important components of disease epidemiology in production environments.

### **Concluding remarks**

Parasite coinfections predominate in host populations in the wild and can have significant implications for key parasite traits such as virulence [1]. Most coinfections, however, do not occur simultaneously, but sequentially with one parasite establishing first, which can change the outcome of infection and epidemiology of a disease. We argue that the prevalence and significance of sequential coinfections for ecological and evolutionary dynamics of host-parasite interactions is probably largely underestimated in the wild. While interactions are generally considered more likely between related parasites, recent evidence has begun to reveal sequential coinfection interactions also between taxonomically distant parasites. This could significantly increase the complexity of possible interactions within the parasite community of one host. However, the magnitude of possible interactions is still largely unknown (see

Outstanding Questions). Sequential coinfection interactions are also influenced by different types of direct and indirect interactions between the parasites. In most cases, however, detailed mechanisms have remained unknown. Particularly the role of specific immune responses of vertebrates is still poorly understood although these responses probably shape subsequent parasite interactions long after the primary infection itself has been cleared. It would also be important to explore the evolutionary implications of sequential coinfections in detail. For example, a sequence between two infections generally attenuates virulence, but the variation associated in specific genotype interactions (G×G) between the coinfecting parasites is largely unknown. Such information could help elucidate which virulence genotypes are favoured by selection. Further, simultaneous coinfections could facilitate transmission of the coinfecting partners. This could impose selection on transmission strategies, but also depend on the sequential infection history and immunological status of the hosts. In general, aspects of sequential host exposure are important also from an applied perspective as sequential epidemics of different pathogens are common also in intensive production environments. Acknowledgement and integration of dynamics of infections in implementation of management practices would be essential in fight against emerging parasitic diseases and drug resistance.

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*Glossary*

**Coinfection:** infection of a host by more than one parasite; syn. multiple infection, concomitant infection.

**Fitness:** number of descendants of an individual related to the number of descendants of other individuals in a population

**Intermediate host:** a host that transmits the infection to the next host (another intermediate host or a definitive host) in a complex parasite life cycle. Parasites can use intermediate hosts for growth and/or asexual reproduction.

**Transmission:** movement of a parasite between host individuals; can take place horizontally through direct contact between hosts (e.g. bacteria, viruses, free-living infective stages) or via vectors and intermediate hosts (e.g. many parasitic worms), or vertically from mother to offspring.

**Virulence:** the magnitude of negative impact of a parasite on its host, often measured as reduction in host fecundity or lifetime.

**Outstanding Questions**

How widely do effects of sequential coinfections extend across different parasite taxa? Do these operate on the scale of the entire parasite community of one host?

What are the detailed mechanisms by which sequentially coinfecting parasites interact within a host? How do these differ across different hosts such as plants, invertebrates and vertebrates and between different components of the host immune system?

Are sequential coinfections important for evolution of parasite virulence and transmission strategies?

What is the significance of coinfections and sequential coinfections for disease severity in production environments? Can knowledge of ecology and evolution of sequential infections provide tools for disease control?

Table 1. Examples of plant, invertebrate and vertebrate systems demonstrating effects of sequential parasite coinfections.

<b>Host</b>	<b>Coinfecting parasites</b>	<b>Outcome of sequential infection</b>	<b>Possible mechanism</b>	<b>Reference</b>
White campion, <i>Silene latifolia</i>	Strains of anther smut fungus, <i>Microbotryum violaceum</i>	First arriving strain has an advantage over later arriving strains	Competitive exclusion by an unknown mechanism	[5]
Ribwort plantain, <i>Plantago lanceolata</i>	Strains of powdery mildew, <i>Podosphaera plantaginis</i>	First infection provides protection against later strains, but results in higher infection later in the season	Apparent (host-immune mediated) competition	[3]
Tomato plant ( <i>Solanum</i> sp.) epidermal cells	Strains of powdery mildew, <i>Oidium neolycopersici</i>	An avirulent strain suppressed a virulent strain	Hypersensitive reaction at the scale of single cells	[51]
Brown mustard, <i>Brassica juncea</i>	Compatible and incompatible strains of oomycete, <i>Albugo candida</i>	Incompatible strain induces protection against the compatible strain	Durable, systemic immune response	[52]
Barley, <i>Hordeum vulgare</i>	Barley stripe mosaic virus and barley yellow dwarf virus	Lower virulence compared to simultaneous infection; lower concentration of	Apparent (host-immune mediated) or interference competition, details unknown	[4]

		the later arriving virus		
Waterflea, <i>Daphnia magna</i>	Strains of the bacterium <i>Pasteuria ramosa</i>	More competitive and virulent strains dominate, except when a less-virulent strain infects first	Apparent (host-immune mediated) or interference competition, details unknown	[53]
Waterflea, <i>Daphnia magna</i>	Bacterium <i>Pasteuria ramosa</i> and microsporidium <i>Octosporea bayeri</i>	No effect, but <i>O. bayeri</i> was able to withstand competition when first infected host vertically	Competition by an unknown mechanism	[28]
Waterflea, <i>Daphnia galeata</i>	Protozoan <i>Caullerya mesnili</i> and fungus <i>Metschnikowia</i> sp.	Higher prevalence of coinfection, <i>C. mesnili</i> suppressing <i>Metschnikowia</i> sp.	Apparent (host-immune mediated) or interference competition, details unknown	[6]
Honeybee, <i>Apis mellifera</i>	Microsporidia <i>Nosema apis</i> and <i>N. ceranae</i>	Species infecting first inhabits the second; magnitude of the effect depends on the species	Apparent (host-immune mediated) or resource competition, details unknown	[7]
Pacific chorus frog, <i>Pseudacris regilla</i>	Trematodes <i>Ribeiroia ondatrae</i> and <i>Echinostoma trivolvis</i>	Success of <i>R. ondatrae</i> is reduced by <i>E. trivolvis</i> ; no effect in opposite order of infections	Apparent (host-immune mediated) or resource competition, details unknown	[10]
Laboratory mouse	Strains of rodent malaria,	Reduction in density of the later	Apparent (host-immune mediated)	[11]

	<i>Plasmodium chabaudi</i>	arriving strains with the length of sequence between infections	or resource competition, details unknown	
Laboratory mouse (meta-analysis on 54 studies)	Metazoan parasitic worms and microparasites	No effect of infection interval; decreased or increased microparasite densities in coinfection	Anemia and immune-suppression of the host resulting in decreased or increased microparasite densities, respectively	[12]
African buffalo, <i>Syncerus caffer</i>	Gastrointestinal nematode and bovine tuberculosis ( <i>Mycobacterium bovis</i> ; TB)	Prior nematode infection facilitates the invasion of bovine TB	Nematode-induced immune-suppression	[48]

Box 1. Coinfecting parasites are generally considered competitors for limited host resources. These interactions can take place directly in the form of interference competition, or indirectly as competition for resources or through apparent host immune-mediated competition. In sequential coinfections, one of the parasites invades the host first and the timing between the two infections can vary from few moments to years. This can modify the interactions, for example, the first parasite gaining a competitive advantage by taking over host resources before the second invader or eliciting cross-reactive host immune responses that suppress the later arriving parasite (Fig. I). It is also important to note that the effects of infection sequence can depend on which parasite infects the host first, i.e. the effects can be asymmetrical (in Fig I, blue parasite can infect before red, or vice versa). In general, sequential infection can result in competitive exclusion without coexistence (superinfection, see [32]) with only a small or no effect on the performance of the first invader. In a common scenario, sequentially establishing parasites coexist, but the first infection suppresses the second one, which typically results in lower virulence compared to a simultaneous coinfection (Fig. I). Sequential infection effects can also arise when the first infection becomes cleared by the host, but cross-reactive immune responses elicited by the first parasite influence the success of the later infections in absence of actual coexistence. Simultaneous coinfection can also facilitate parasite infection success, for example, if the genetically diverse infection represents a higher challenge to the host immune system compared a single-infection. In sequential coinfection, however, such facilitation may be reduced if the host has already mounted an immune response consequently to a previous infection from the same or different parasite (see also Box 2). In an opposite scenario, sequential infection results, for example, in immunosuppression of the host allowing higher replication of the second parasite [12]. Overall, these scenarios can also be influenced by parasite within-host dynamics (e.g. acute vs. chronic and local vs. systemic infections) as well as parasite taxonomic relatedness, which exemplifies the complexity of possible interactions in a coinfecting parasite community.

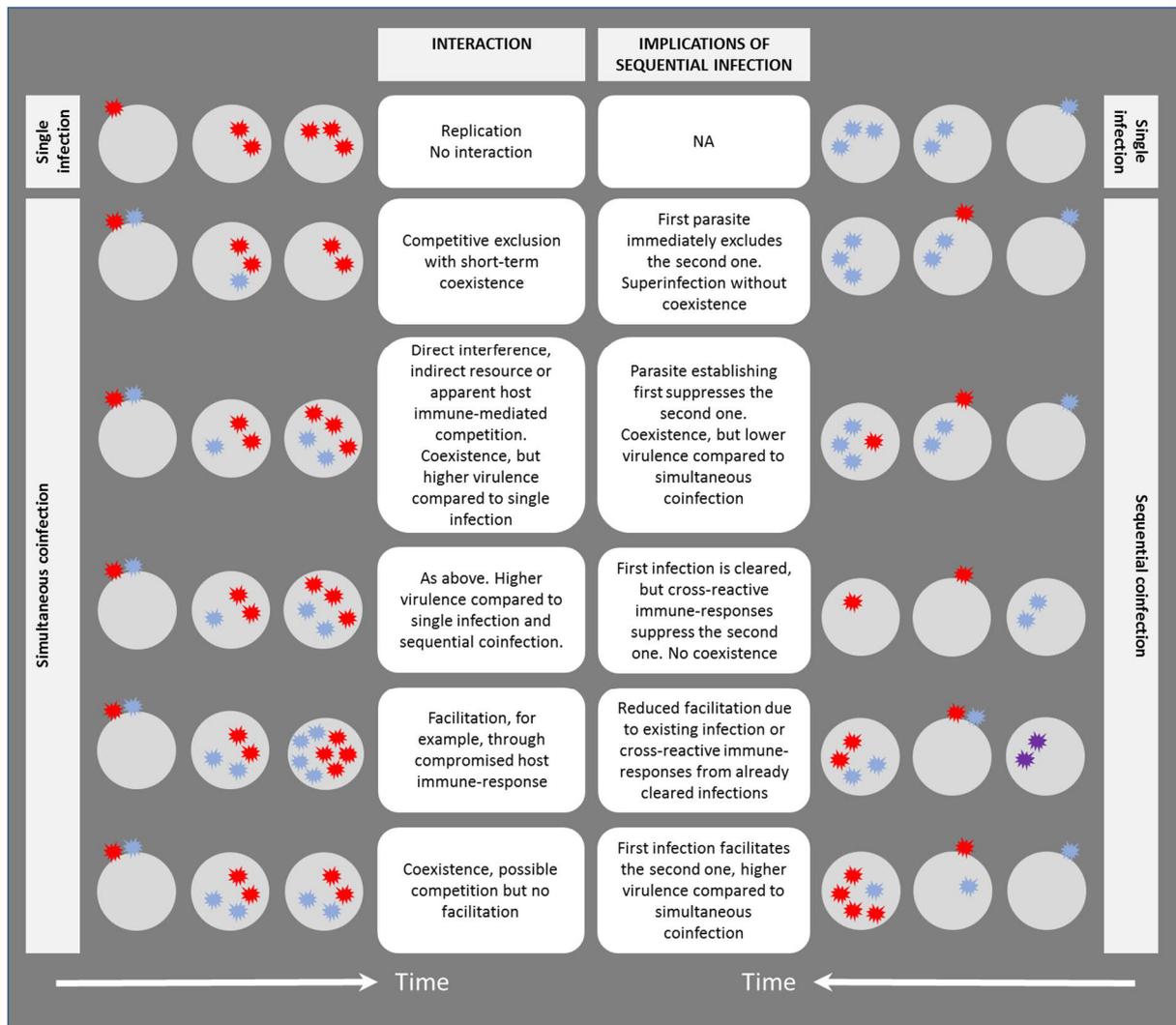


Fig I. Schematic presentation of possible interactions and implications of simultaneous (left) and sequential (right) parasite coinfections in a host. Parasite strains / genotypes are denoted in different colors.

Box 2. Trematodes of the genus *Diplostomum* are ubiquitous parasites of freshwater fishes with several species [2] and genotypes [54] of the parasites typically coinfecting a host. Different coinfecting species interact in fish in genotype-specific and dose-dependent manner, which changes the infection success of the parasites [46]. Cercariae are released to water in high numbers from the first intermediate freshwater snail hosts after asexual reproduction. One snail can harbor and release more than one clonal parasite genotype, which can result in simultaneous co-exposure of the fish to two genotypes. Similarly, a fish can be simultaneously co-exposed to two parasite genotypes emerging from two different snails if the snails are in close proximity (Fig. II). These scenarios are possible in the wild because of aggregation of snail intermediate hosts that release the infective stages (cercariae) in shallow areas of a lake, high prevalence of infection in some populations, and aggregation (coinfection) of parasite genotypes to certain snail intermediate host individuals [55]. Simultaneous co-exposure of a previously unexposed fish to two genotypes (Fig. II) result in higher infection success compared to single-genotype exposures. This is likely as a result of exposure heterogeneity representing an additional challenge to the host immune system in the naïve hosts [23]. The results suggest that co-exposing the fish host could be beneficial to the parasite. However, the benefit is reduced or even eroded if the fish host has been previously exposed [9] (Fig. II), presumably following development of specific immune responses in the fish (the immunization process itself is not specific to parasite genotypes [56]). Thus, after likely activation of host adaptive immune system following the first exposure, parasites no longer benefit from co-exposing a host.

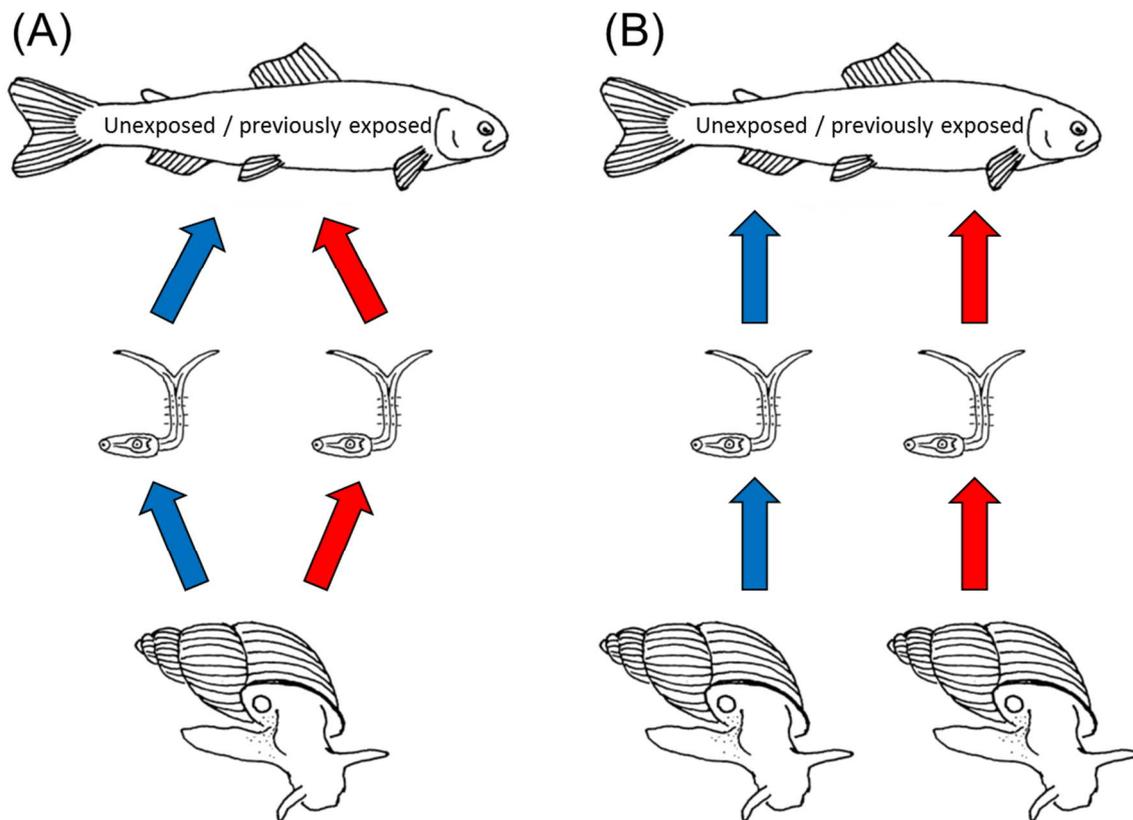


Fig II. Scenarios of simultaneous and sequential coinfections of a fish host by *Diplostomum pseudospathaceum* trematode cercariae. Simultaneous co-exposure of fish can result from one snail releasing two parasite genotypes (denoted by different colors) (A), or from two different snails releasing single genotypes in close proximity (B). Infection success and facilitation of the genotypes depends whether the fish is unexposed or has been previously exposed (sequential exposure). Drawings courtesy of Sven Nikander.

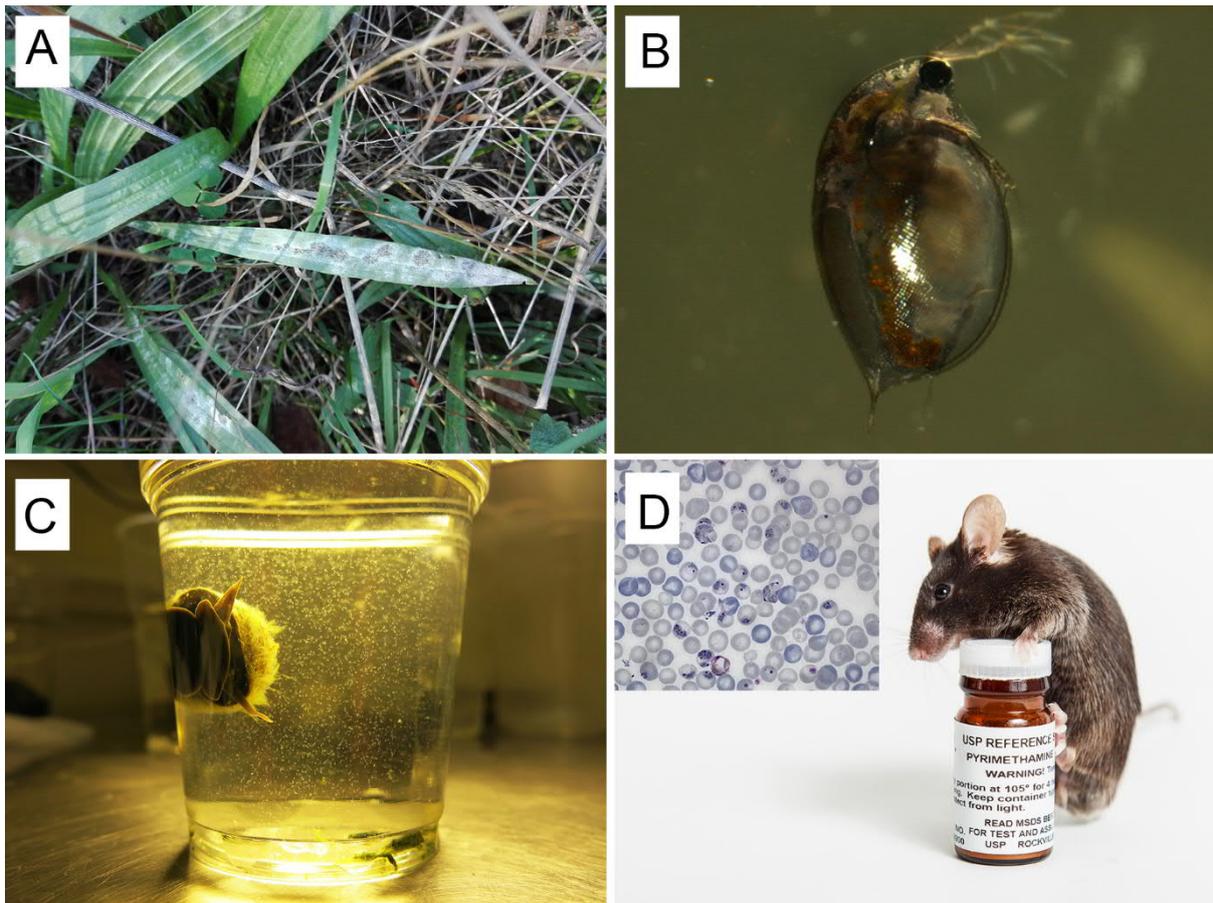


Fig. 1. Examples of systems where effects of sequential parasite coinfections have been explored. Sequential infection of *Plantago lanceolata* with a strain of the powdery mildew *Podosphaera plantaginis* can provide protection against later arriving strains of the same pathogen during early growing season [3] (A; photo shows whitish fungal lesions on the leaves of *P. lanceolata*, photo by Anna-Liisa Laine). A prior residency of low-virulent strains of *Pasteuria ramosa* in *Daphnia* decreases virulence of high-virulent strains [53] (B; photo shows infection of *P. ramosa* in *D. longispina* seen as whitish formations behind and under the eye, courtesy of Katja Pulkkinen). Two genotypes of *Diplostomum pseudospathaceum* clonal cercariae released from the same snail intermediate hosts are more infectious than single genotypes, but only if the host has not encountered the parasite earlier [9] (C; photo shows a dense swarm of cercariae released from an infected snail, photo by Anssi Karvonen). Competitively inferior strains of the rodent malaria *Plasmodium chabaudi* can gain a competitive advantage over competitively superior strains by infecting the mouse host first [11] (D; photos courtesy of Andrew Read and Sarah Reece).

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