On multiple infections by parasites with complex life cycles

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Abstract

Host manipulation is a common strategy of parasites of different complexity. Host manipulation directly affects predator-prey dynamics in trophically transmitted parasites, where parasite transmission requires predation. Theoretical studies suggest that manipulation that enhances predation often results in a heavy burden on the prey population. Consequently, the system is often destabilised, making parasites prone to extinction. Host manipulation, however, can also suppress predation. Such suppression is possible if multiple parasites coinfect a host with conflicting interests in manipulation. The interests could be misaligned for various reasons, such as limited carrying capacity or parasitoid developmental stage. Multiple infections are a norm in parasite ecology but are often neglected in the theoretical assessment of host-parasite dynamics. We tease apart the effect of host manipulation of coinfected parasites and manipulation interests via a mathematical model of a trophically transmitted parasite with a complex life cycle. The life cycle comprises a free-living state, an intermediate and a definitive host. With coinfection, we show that host manipulation that enhances predation need not permanently destabilise the predator-prey system. However, cooperation between coinfected parasites leading to increased predation and can

~~lead to~~ sabotage in manipulation can induce bistability such that a slight disturbance in the system drives the parasite population to extinction. ~~On the other hand, when coinfected parasites sabotage the manipulative ability of one another, the stabilityof the predator-prey system is always guaranteed~~Intriguingly, cooperation in both aspects, host manipulation and reproduction, might ensure system stability. In some cases, a lack of cooperation in reproduction may actually prevent the dynamical system from bistability. Our study highlights the necessity and means of incorporating the reality of multiple parasites and their multi-trophic life cycles in a single system in studying parasite ecology.

Introduction

Parasites infect life on earth ubiquitously, and many of these parasites have complex life cycles (Zimmer, 2001). While a complex lifecycle can be defined as abrupt ontogenic changes in morphology and ecology (Benesh, 2016), a complex parasitic lifecycle typically involves numerous hosts that a parasite needs to traverse to complete its life cycle. This complex lifecycle results in the evolution of various strategies that enable the success of parasite transmission from one host to another. One famous strategy that inspires many science fiction movies and novels is host manipulation, where a parasite can alter the morphology and/or behaviour of its host to enhance its transmission to the next host (Hughes et al., 2012). Host manipulation has been shown in many host-parasite systems, from parasites with simple life-cycle to those with complex life-cycle that involves more than one host (Hughes et al., 2012; Molyneux and Jefferies, 1986). For instance, sand flies infected by *Leishmania* parasites bite more and take more time for a blood meal from mammals (the definitive host of *Leishmania*) compared to their uninfected counterparts (Rogers and Bates, 2007). Copepods infected by cestode parasites are more active and accessible to sticklebacks (the definitive hosts of the cestodes) compared to uninfected copepods (Wedekind and Milinski, 1996).

Theoretical studies have long attempted to understand the ecological and evolutionary consequences of host manipulation. Roosien et al. (2013) and Hosack et al. (2008) showed that manipulative parasites could increase the disease prevalence in an epidemic. Gandon (2018) studied the evolution of the manipulative ability of infectious disease parasites, showing different evolutionary outcomes depending on whether the pathogen can control its vector or host. Hadeler and Freedman (1989); Fenton and Rands (2006) and Rogawa et al. (2018) showed that host manipulation could stabilise or destabilise the predator-prey dynamics depending on how manipulation affects the predation response function and the assumption on the fertility of the definitive infected host. Seppälä and Jokela (2008) showed that host

manipulation could evolve even when it increases the risk of the intermediate host being eaten by a non-host predator, given that the initial predation risk is sufficiently low. These models, however, lack a crucial aspect of parasite dynamics, multiple infections (Kalbe et al., 2002)

Typical studies do not consider multiple infections, a phenomenon that is the norm rather than an exception in parasitism. Multiple infections result in the coinfection of more than one parasite inside a host, which may alter the manipulative outcomes (figure 1). An alignment of interest between coinfecting parasites may enhance manipulation, while a conflict of interest may reduce the manipulative effect. Indeed, Hafer and Milinski (2015) showed that copepods infected by two cestode parasites reduce the activity of copepods when both parasites are at the same noninfectious stage, i.e. both parasites are not ready to transmit. Thus the reduction in mobility is suggested to reduce the predation rate by the definitive hosts. When two infectious parasites infect the copepods, the copepods' activity increases, and so does the predation risk for the copepod. However, when the copepods are infected by one infectious and one noninfectious parasite, their interests clash, and one parasite wins over the other.

Theoretical work that considers multiple infections often focuses on the evolution of virulence (van Baalen and Sabelis, 1995; Alizon et al., 2013; Alizon and van Baalen, 2008; Choisy and de Roode, 2010; Alizon, 2012). They show that multiple infections can increase virulence (van Baalen and Sabelis, 1995; Choisy and de Roode, 2010). Evolutionary branching of a less virulent and a hypervirulent parasite can occur when within-host dynamics are considered (Alizon and van Baalen, 2008), and a reduction in virulence is possible if parasites are co-transmitted (Alizon, 2012). ~~In epidemiological models, higher virulence often assumes a link with a higher transmission rate ; virulence is therefore associated with host manipulation in such cases~~As host manipulation is suggested to affect transmission rate, these studies also involve host manipulation to a certain extent even though they do not explicitly consider the trait. Host manipulation in trophically transmitted parasite receive less attention. Although manipulation is correlated with transmission rate in both trophically transmitted parasites and infectious disease, there are subtlely difference. Host manipulation influences the predation rate in trophically transmitted parasites, predominantly affecting predator-prey dynamics. Theoretical studies on host manipulation in trophically transmitted parasites with multiple infections are rare (Parker et al., 2003; Vickery and Poulin, 2009). Moreover, they do not consider the prey-predator dynamics, which will likely have important feedback on the evolution of host manipulation. A few studies considering the prey-predator dynamics do not incorporate multiple infections (Rogawa et al., 2018; Iritani and Sato, 2018; Hadeler and Freedman, 1989; Fenton and Rands, 2006). More importantly, they assume that transmission from definitive hosts to intermediate hosts is due to direct contact between the

Figure 1: **Who is in control?.** Schistocephalus eggs, which overwinter at the bottom of bodies of water, hatch into microscopically small swimming larvae. These larvae are eaten by copepods (also known as Cyclops due to its single eye), where they develop to the second larval stage. However, the copepod is only the first intermediate host. The larvae are then eaten by sticklebacks, where they reach the third larval stage and grow significantly in size and weight. For the parasite to successfully reach its final host, a warm-blooded animal like a bird, it manipulates its intermediate hosts. The timing is crucial as the chances of success are greatest if the larvae develop in the copepod for 13 to 15 days before entering the stickleback. The presence of multiple parasites in the same host can lead to competition and strategic decision pertaining to investment in manipulation and growth. And indeed a stickleback can be infected by numerous tapeworms as shown above by Martin Kalbe.

two types of hosts. This is often not the case, as parasites are released from the definitive hosts into the environment. Transmission happens only when intermediate hosts have contact with this free-living parasite pool.

Our study addresses the gap in the theoretical work on host manipulation in trophically transmitted parasites. We include multiple infections and consider the dynamics of the free-living parasite pool. Our compartment model helps illustrate a parasite's complex lifecycle with two hosts: an intermediate host preyed upon by a definitive host. Transmission from the intermediate host to the definitive host occurs when predation on infected intermediate hosts happens. Reproduction only happens in the definitive hosts. New parasites are then

released into the environment, where they again have contact with the intermediate hosts to complete their lifecycle. We focus on the manipulation of the intermediate hosts, such that the parasite increases the predation rate on the intermediate host by the definitive host to increase its transmission rate. We then analyse the effect of host manipulation on the ecological dynamics in the prey-predator-parasite system. In contrast to the examples mentioned above our model consists of a single intermediate host as it already provides enough complexity to discuss between transmission and manipulation. We found that ~~cooperation~~ sabotage in host manipulation ~~leads to bistability in the predator-prey system , given that reproduction from multiple infections is sufficiently high. This finding~~ almost always pushes the dynamical system toward bistability, provided that the reproduction in single infection is sufficiently small. The bistable nature suggests that the predator-prey parasite system is finely balanced and susceptible to extinction via ecological disturbances. Initially ~~surprising, we show how sabotage in host manipulation guarantees a unique stable equilibrium in the system~~supprising, we showed that cooperation in both host manipulation and reproduction is not always beneficial and might expose the parasite population to the risk of extinction.

Model and Results

Our model concerns the complex lifecycle of a trophically transmitted parasite that requires two hosts: an intermediate host and a definitive host. Reproduction only happens inside the definitive hosts, releasing new parasitic progeny in the environment. An intermediate host can be infected if it encounters this free-living parasite pool. Finally, when a definitive host consumes an infected intermediate host, the definitive host gets infected, and the parasite completes its lifecycle.

For simplicity, we assume that hosts can be infected by one (single infection) or, at most, two parasites (double infections). Our model is, therefore, more relevant to the macroparasitic system. Given that infection occurs, the probability that two parasites from the parasite pool co-transmit to an intermediate host is denoted by p. Thus $1-p$ is the probability that a single parasite enters an intermediate host. When a definitive host consumes an intermediate host infected by two parasites, there is a probability q that the parasites co-transmit to the definitive host. With probability $1-q$, only one parasite successfully transmits. This formulation assumes that infection always happens when hosts encounter parasites. The dynamics of a complex lifecycle parasite that requires two hosts is described by the following system

of equations, firstly for the intermediate host as,

$$\begin{aligned}
\frac{dI_s}{dt} &= R(I_s, I_w, I_{ww}) - dI_s - P_s(D_s, D_w, D_{ww})I_s - \eta I_s \\
\frac{dI_w}{dt} &= (1-p)\eta I_s - (d+\alpha_w)I_w - P_w(D_s, D_w, D_{ww}, \beta_w)I_w \\
\frac{dI_{ww}}{dt} &= p\eta I_s - (d+\alpha_{ww})I_{ww} - P_{ww}(D_s, D_w, D_{ww}, \beta_{ww})I_{ww}
\end{aligned} \tag{1}$$

where $R(I_s, I_w, I_{ww})$ represents the birth rate of the intermediate hosts, a function of both infected and uninfected individuals. P_s, P_w, P_{ww} are the predation functions of definitive hosts on susceptible, singly infected and doubly infected intermediate hosts. The predation function depends on the density of the definitive hosts and the manipulative strategies of parasites in the intermediate hosts. In particular, if a single parasite infects an intermediate host, the manipulation strategy is β_w. However, if the intermediate host is co-infected, the manipulation strategy is β_{ww}. In the scope of this model, we assume no specific relationship between β_w and β_{ww} to explore all possible ecological outcomes of the system. The force of infection by parasites in the environment is denoted by $\eta = \gamma W$. Since parasites can manipulate intermediate and definitive hosts, here, whenever we mention host manipulation, it specifically refers to the manipulation in intermediate hosts, which correlates to the predation rate.

For the definitive hosts we have,

$$\begin{aligned}
\frac{dD_s}{dt} &= B(D_s, D_w, D_{ww}, I_s, I_w, I_{ww}) - \mu D_s - (2\lambda_{ww} + \lambda_w)D_s \\
\frac{dD_w}{dt} &= (\lambda_w + 2(1-q)\lambda_{ww})D_s - (\mu + \sigma_w)DwD_w - (2(1-q)\lambda_{ww} + \lambda_w)D_w \\
\frac{dD_{ww}}{dt} &= 2q\lambda_{ww}D_s + (2(1-q)\lambda_{ww} + \lambda_w)D_w - (\mu + \sigma_{ww})D_{ww}
\end{aligned} \tag{2}$$

where $B(D_s, D_w, D_{ww}, I_s, I_w, I_{ww})$ represents the birth rate of definitive hosts. The birth rates depend on the density of both intermediate and definitive hosts, infected or uninfected. The force of infection that corresponds respectively to singly infected intermediate host (I_w) and doubly infected intermediate hosts (I_{ww}) is denoted respectively by ~~$\lambda_w = \beta_w I_w$~~ and ~~$\lambda_{ww} = \beta_{ww} I_{ww}$~~. $\lambda_w = h_1(\rho + \beta_w)I_w$ and $\lambda_{ww} = h_2(\rho + \beta_{ww})I_{ww}$, where ρ is the based line predation rate and h_1 and h_2 are the probability that the parasite successfully established inside the host. If there is no manipulation, that is, $\beta_w = \beta_{ww} = 0$, the parasite is still transmitted via the based line predation. The dynamics of the free-living parasites in the

environment are then given by,

$$\frac{dW}{dt} = f_w D_w + f_{ww} D_{ww} - \delta W - \eta I_s. \tag{3}$$

Definitions of different parameters can be found in Table 1.

Here, we focus on manipulation that enhances transmission from intermediate hosts to definitive hosts; we thus simplify the transmission from the parasite pool to intermediate hosts such that no sequential infection occurs at this transmission state. This assumption may not be implausible, given that the prey' lifecycle is often shorter than that of the predator. A prey likely encounters the free-living parasite pool once and then either die due to predation, making sequential transmission less likely at this state. Sequential infection can happen when parasites transmit from intermediate hosts to definitive hosts. Therefore, a singly infected definitive host can be further infected by another parasite if it consumes infected intermediate hosts. The system's dynamics are illustrated in figure (2).

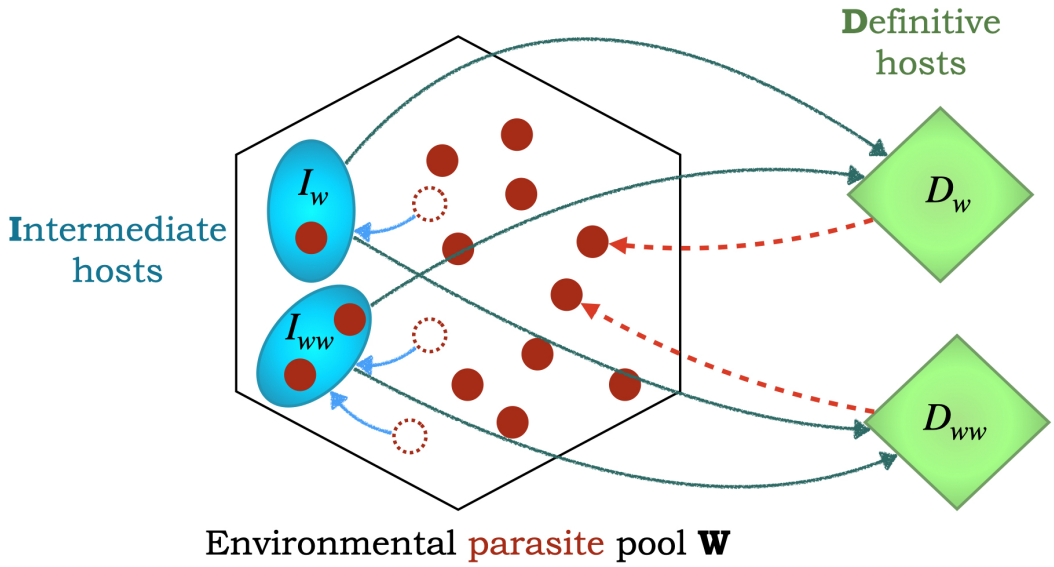

Figure 2: Schematic of the model. Blue ovales represent intermediate host compartment, green diamonds represent definitive host compartment, and the transparent hexagon represents the parasite pool compartment with red circles illustrating individual parasites.

Basic reproduction ratio R_0 of the parasites

The basic reproduction ratio R_0 (or basic reproduction number as often used in epidemiology) indicates parasite fitness. It can be understood as the expected number of offspring a parasite produces during its lifetime when introduced to a susceptible host population. We calculate the basic reproduction ratio R_0 using the next-generation method (Diekmann et al., 1990, 2009; Hurford et al., 2010) (See SI1 for details).

$$R_0 = \overbrace{\gamma I_s^* \frac{2pqh(\rho+\beta_{ww})}{\alpha_{ww}+d+P_{ww}} \frac{D_s^*}{\mu+\sigma_{ww}} \frac{f_{ww}}{\delta+\gamma I_s^*}}^{\texttt{Double infections}} + \underbrace{\gamma I_s^* \left(\frac{(1-p)h(\rho+\beta_w)}{\alpha_w+d+P_w} + \frac{2p(1-q)h(\rho+\beta_{ww})}{\alpha_{ww}+d+P_{ww}} \right) \frac{D_s^*}{\mu+\sigma_w} \frac{f_w}{\delta+\gamma I_s^*}}_{\texttt{Single infection}} \tag{4}$$

where I_s^* and D_s^* are the densities of susceptible intermediate and definitive hosts at the disease-free equilibrium. Here, the expression of R_0 contains the possible reproduction routes of a parasite, which can be via double or single infections. The first component corresponds to the double infections route, in which the focal parasite co-transmits with another parasite into a susceptible intermediate host, then co-transmits into a susceptible definitive host and reproduces. Here, parasites are so rare that only co-transmission matters and the compartments with sequential infections are therefore neglected. The second component corresponds to the single infection route, wherein the focal parasite infects a susceptible intermediate host via single or double infections. The parasite then transmits alone into the susceptible definitive host and eventually reproduces.

If $R_0 > 1$, a parasite spreads when introduced into the disease-free equilibrium of prey and predator. Intuitively, the higher the density of susceptible intermediate and definitive hosts, the larger the value of R_0 as the infection reservoir is more extensive. In contrast, regardless of the explicit form of the predation function, the higher the predation rate P_w and P_{ww}, the lower the value of R_0 given the smaller reservoir of intermediate hosts. The effect of host manipulation on the value of R_0 is not so straightforward; as host manipulation becomes efficient, the transmission rate from the intermediate host to the definitive host increases, but so does the predation rate. A higher predation rate results in a smaller intermediate host reservoir available for the parasites to infect. To understand the effect of manipulation on parasites' fitness and the system's ecological dynamics, we next specify the predation

functions. We consider linear functions for predation to begin with,

$$P_s(D_s, D_w, D_{ww}) = \rho D_{total}$$
$$P_w(D_s, D_w, D_{ww}, \beta_w) = (\rho + \beta_w) D_{total}$$
$$P_{ww}(D_s, D_w, D_{ww}, \beta_{ww}) = (\rho + \beta_{ww}) D_{total}$$

where $D_{total} = D_s + D_w + D_{ww}$ is the total density of the definitive hosts, and ρ is the baseline capture rate of the predator on the prey. If an intermediate host is infected, it is captured by the definitive hosts with rate $\rho + \beta_w$ if it is singly infected and with rate $\rho + \beta_{ww}$ if it is doubly infected. Zero values for β_w and β_{ww} suggest no manipulation, and predation is at the baseline value ρ.

For simplicity, we also consider a linear function of the birth of definitive hosts

$$B(D_s, D_w, D_{ww}, I_s, I_w, I_{ww}) = \rho c D_{total} I_{total}$$

where c is the efficiency of converting prey into predator's offspring, and $I_{total} = I_s + I_w + I_{ww}$ is the total density of the intermediate hosts. It is important to note that host manipulation affects the population dynamics via its influence on predation rate but not the physiological aspect of the definitive host, i.e. the predator. The birth rate of the predators thus depends on the capture rate, but it is not affected by host manipulation, as to our best knowledge, there is no supporting evidence to necessarily consider otherwise.

The explicit form of I_s^* and D_s^*, capturing the predator-prey dynamics, depends on the precise form of all birth and predation functions B, R, P_s, P_w and P_{ww}. But, it does not depend on the manipulation ability or any other parameter of the parasite. Given that the birth rate of the predator and the predation rate are linear functions in prey and predator density, the form of the birth rate R of the prey has a significant effect on the susceptible intermediate and definitive host dynamics. ~~Hence we explore the exact structure of these birth functions next.~~

~~Linear birth~~ Birth function of intermediate hosts

~~Here, we consider the system when the birth function R of the intermediate host is linear, specifically, $R(I_s, I_w, I_{ww}) = rI_{total}$. The equilibrium of intermediate and definitive hosts in~~

~~the disease-free state are,~~

$$I_s^* = \frac{\mu}{c\rho}$$

$$D_s^* = \frac{r-d}{\rho}$$

~~This~~

The simplest form of the prey's birth rate is a linear function, in which case the disease free equilibrium is always unstable. In particular, it has a cyclic behaviour because, at this equilibrium, the jacobian matrix of the system (1, 2, 3) always has two pure imaginary eigenvalues (see SI2). This follows from the Lotka-Volterra system using linear functions for prey birth and predation (Lotka, 1920). Since the disease-free dynamics is cyclic, it is difficult to analyse the spread of a parasite ~~(often~~ using the basic reproduction ratio, which is evaluated when the disease-free state is stable~~)~~. Here, $R_0 > 1$ happens when γ, the transmission rate from the environment to intermediate hosts, and the reproduction rates f_w, f_{ww} are significantly large (the specific mathematical conditions can be found in ~~SI2~~SI3). However, even when this condition is satisfied, the parasite may not be able to spread and persist in cyclic susceptible host dynamics (Figure 3). This result agrees with the conclusion in (Ripa and Dieckmann, 2013), which suggests that it is difficult for a mutant to invade a cyclic resident population. In our case, it is not the invasion of a mutant in a resident population but the invasion of a parasite in a cyclic disease-free host population; the argument, however, remains valid in both cases. This issue deserves a more thorough investigation, which is out of the scope of this article. ~~We, therefore,~~ Here, we choose a non-linear birth function of the intermediate hosts to obtain a stable disease circulation state and focus on the effect of host manipulation on the ecological dynamics.

~~Non-linear birth function of intermediate hosts~~

~~We chose the~~ The logistic growth for the non-linear birth function ~~, that is~~ follows by

$$R(I_w, I_s, I_{ww}) = rI_{total}(1 - kI_{total})$$

where k is the intraspecific competition coefficient. The disease-free equilibrium is as follows

$$I_s^* = \frac{\mu}{c\rho} \; ; \; D_s^* = \frac{c\rho(r-d) - k\mu r}{c\rho^2}$$

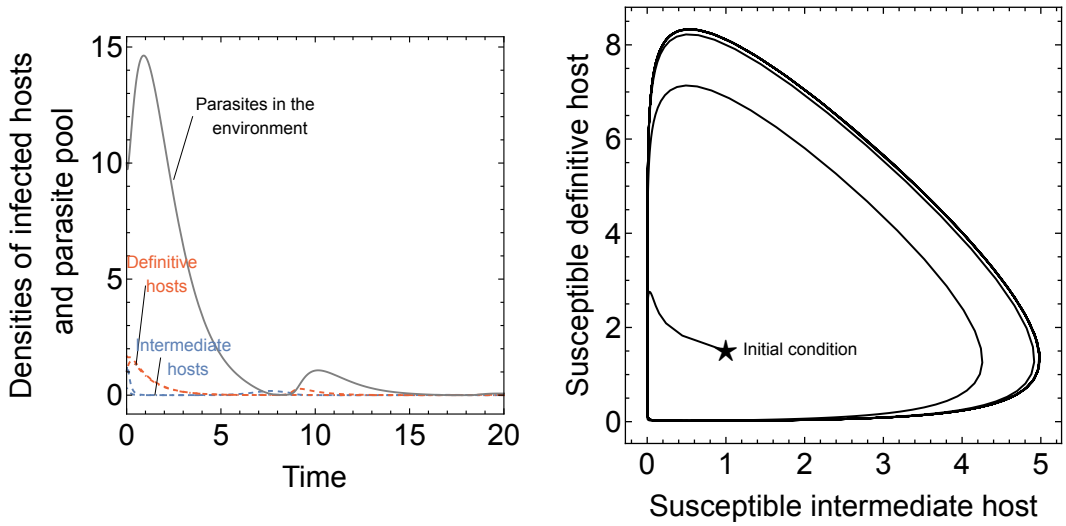

Figure 3: Disease-free equilibrium using linear birth function, where parasite goes extinct (left panel), and susceptible hosts demonstrate cyclic dynamics (right panel). Solid gray line indicate the density of free-living parasites, blue lines indicate infected intermediate hosts while red lines indicate infected definitive hosts. Dashed lines indicate singly infected hosts while dot-dashed lines indicate doubly infected hosts. Parameter values $\rho = 1.2,\ d = 0.9,\ r = 2.5,\ \gamma = 2.9,\ \alpha_w = \alpha_{ww} = 0,\ \beta_w = 1.5,\ \beta_{ww} = 1.5,\ p = 0.1,\ c = 1.4,\ \mu = 0.9,\ \sigma_w = \sigma_{ww} = 0,\ q = 0.01,\ f_w = 6.5,\ f_{ww} = 7.5,\ \delta = 0.9$, ~~$R_0 = 2.233$~~ $h_1 = h_2 = 0.8$, $R_0 = 4.997$

This equilibrium is ~~stable if the following three conditions are met,~~

$$(i)\ \ r > d$$

$$(ii)\ \ \frac{2c\rho\left(\sqrt{\frac{-d+\mu+r}{\mu}} - 1\right)}{r} \leq k < \frac{c\rho(r-d)}{\mu r}$$

$$(iii)\ \ \mu > \frac{4c^2\rho^2 r - 4c^2 d\rho^2}{4ck\rho r + k^2 r^2}.$$

~~The above conditions suggest that (i) the intrinsic reproduction of intermediate hosts r needs to be greater than their natural mortality rate d. More importantly, (ii) the intraspecific competition coefficient has to be within a range allowing the population to survive. Finally, (iii) the definitive host's natural mortality rate must be sufficiently large. Satisfying such~~

conditions, we obtain a stable disease-free equilibrium (positive and stable if components of the parasite, such as reproduction and transmission are sufficiently small, details of the condition can be found in SI4 (Figure 4B).

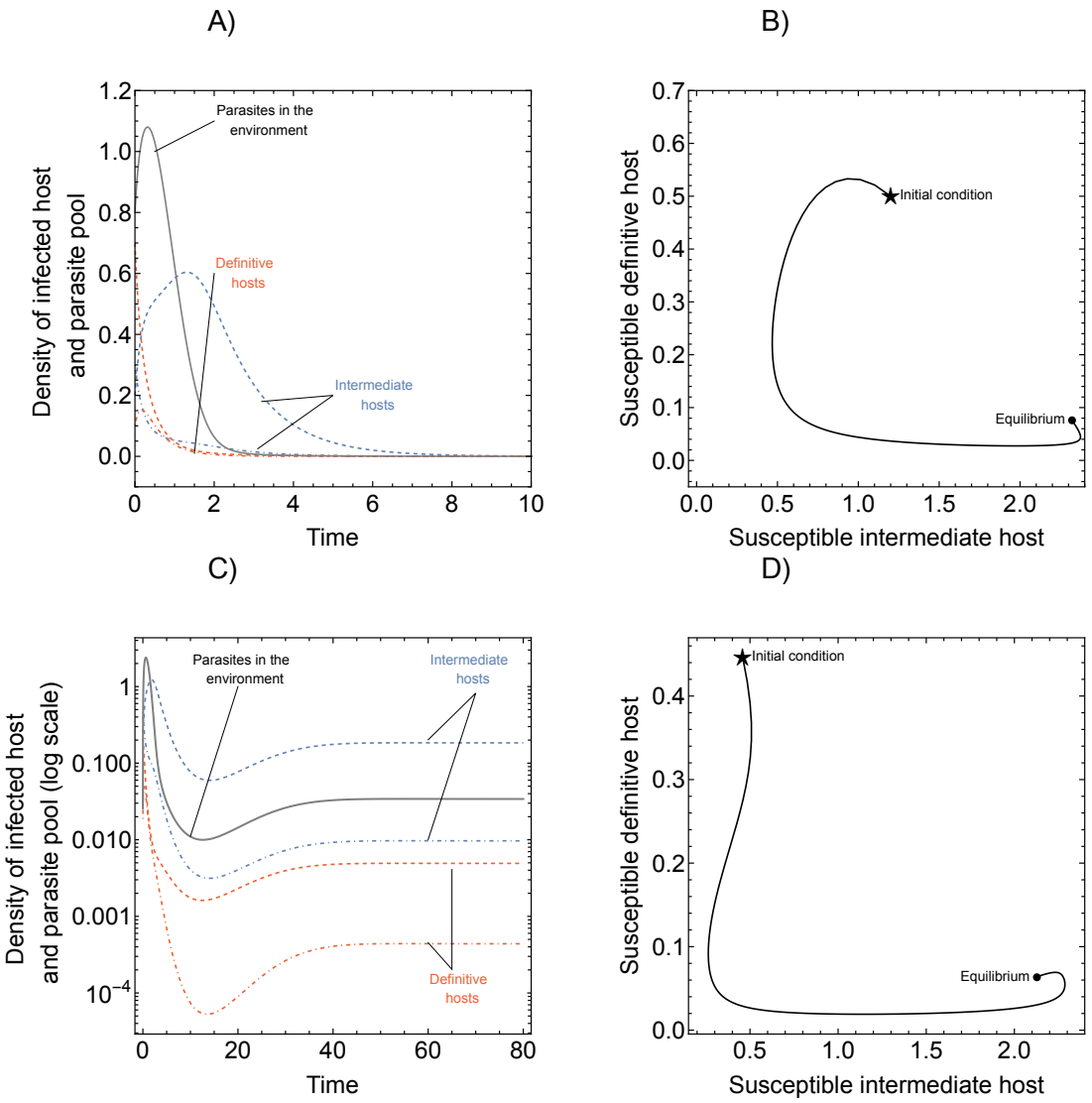

Figure 4: A, B)Disease free equilibrium where parasite cannot persist. C, D) Disease stable equilibrium. Annotations are the same in figure 3. Parameters for disease free equilibrium ~~$\rho = 1.2,\ d = 0.9,\ r = 2.5,\ \gamma = 2.9, \alpha_w = \alpha_{ww} = 0,\ \beta_w = \beta_{ww} = 1.5,\ p = 0.1,\ c = 1.4,\ \mu = 3.9,\ \sigma$~~ Disease stable equilibrium have the same parameter values except for higher host manipulation $\beta_w = \beta_{ww} = 4.5$ and parasite reproduction $f_w = f_{ww} = 45$

When a parasite appears in the disease-free equilibrium, it spreads if its reproduction ratio

$R_0 > 1$. Since the expression is complicated, we could not obtain analytical solutions for this inequality without assumptions. We assume the same parasite virulence, $\alpha_w = \alpha_{ww}$, $\sigma_w = \sigma_{ww}$, and reproduction in double infection as a linear function concerning reproduction in single infections, $f_{ww} = \epsilon f_w$. When $\epsilon > 1$, reproduction in double infections is greater than reproduction in a single infection, whereas $\epsilon \leq 1$, reproduction in double infections is lower or equal to reproduction in a single infection. We found that the parasite can establish if its reproduction value in a single infection f_w is more significant than a threshold (Figure 5,see ~~SI3~~SI5).

Our numerical results show that the parasite reproduction is substantial compared to other parameters (its value is nearly 40 times greater than other parameters). This observation suggests that trophically transmitted parasites must release many offspring into the environment to persist. Interestingly, bistability occurs if the reproduction rate of the parasite in double infections is greater than in the single infection state (Figure 5A, B). In the bistable region, the parasite population can reach a stable equilibrium if the initial density is large enough. In contrast, with sufficient disturbance, the parasite population could go extinct.

The effect of host manipulation on ecological dynamics

Host manipulation can be cooperative; two parasites increase the predation rate on intermediate hosts, or $\beta_{ww} > \beta_w$. However, it can also be uncooperative; that is, the predation rate on doubly-infected intermediate hosts lower than that on singly-infected ones, or $\beta_{ww} < \beta_w$. Cooperation in parasite manipulation does increases the parasite's basic reproduction ratio ~~(~~R_0 ~~)~~ but the manipulation in single infection has a stronger effect on the value of R_0 (Figure 6). Intuitively, if the manipulation in single infection is small, there is not enough transmission and the parasite goes extinct. However, suppose the ability to manipulate the host in a single infection is ~~not strong enough~~just enough for the parasite population to escape extinction but not strong. In that case, cooperation in host manipulation leads to a bistable state of the system. Within the bistable region, the basic reproduction ratio ~~is~~ can be less than one, suggesting that the parasite cannot spread when its manipulative values are within this area of weak manipulation when coinfected. ~~Parasites that can persist in the population may have weak manipulative activity in a single infection but become much more manipulative in coinfection. Likewise, parasites can persist if uncooperative but can manipulate the intermediate hosts effectively when alone.~~

Cooperation between parasites need not be limited to host manipulation. Parasites can cooperate to have a higher reproduction rate in co-infections, i.e. $f_{ww} > f_w$. Likewise, they can compete for resources, so reproduction in double infection is smaller than in single infection. Without any assumption on the relationship between manipulative abil-

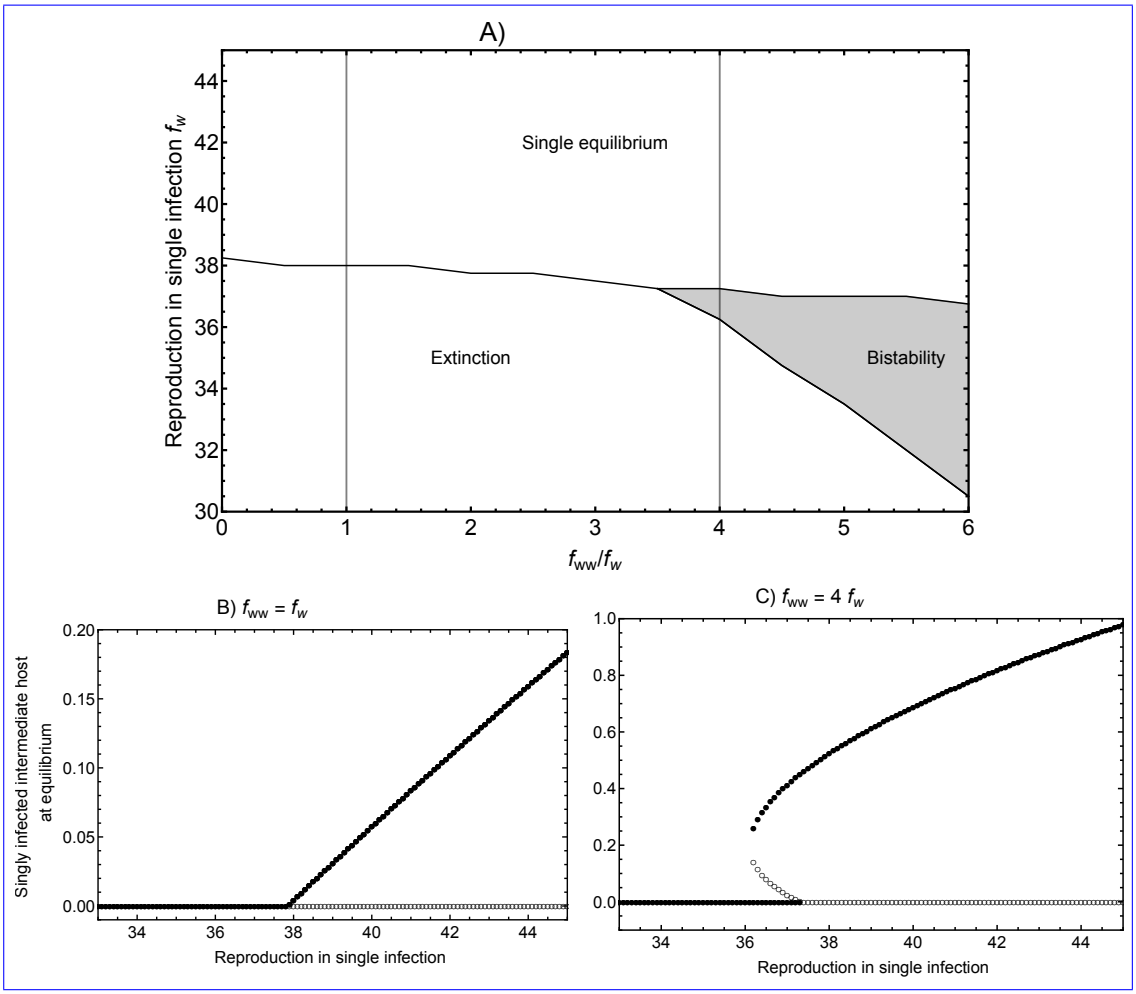

Figure 5: Effect of parasite reproduction on the ecological dynamics. A) Increasing reproduction in doubly infection leads to bistability, B, C) ~~When~~ Density of singly infected host at equilibrium when reproduction of parasites are the same in singly and doubly infected hosts $f_{ww} = f_w$~~. C~~, ~~D) When~~ and when reproduction of parasites in ~~singly~~ doubly infected hosts is four times greater than those in ~~doubly~~ singly infected hosts $f_{ww} = 4f_w$. Filled circles indicate stable equilibrium and open circles indicate unstable equilibrium. Parameter ~~$\rho = 1.2,\ d = 0.9,\ r = 2.5,\ \gamma = 2.9,\ \alpha_w = 0,\ \alpha_w w = 0,\ \beta_w = 1.5,\ \beta_{ww} = 1.5,\ p = 0.1,\ c = 1.4,\ \mu$~~
$\rho = 1.2,\ d = 0.9,\ r = 2.5,\ \gamma = 2.9,\ \alpha_w = 0,\ \alpha_w w = 0,\ \beta_w = 1.5,\ \beta_{ww} = 1.5,\ p = 0.05,\ c = 1.4,$

ity and reproduction, we explore all possible combinations of cooperation and sabotage in both manipulation and reproduction. ~~Interestingly, higher cooperation in manipulation and reproduction enlarges the area of bistability even though it also shrinks the extinction space~~

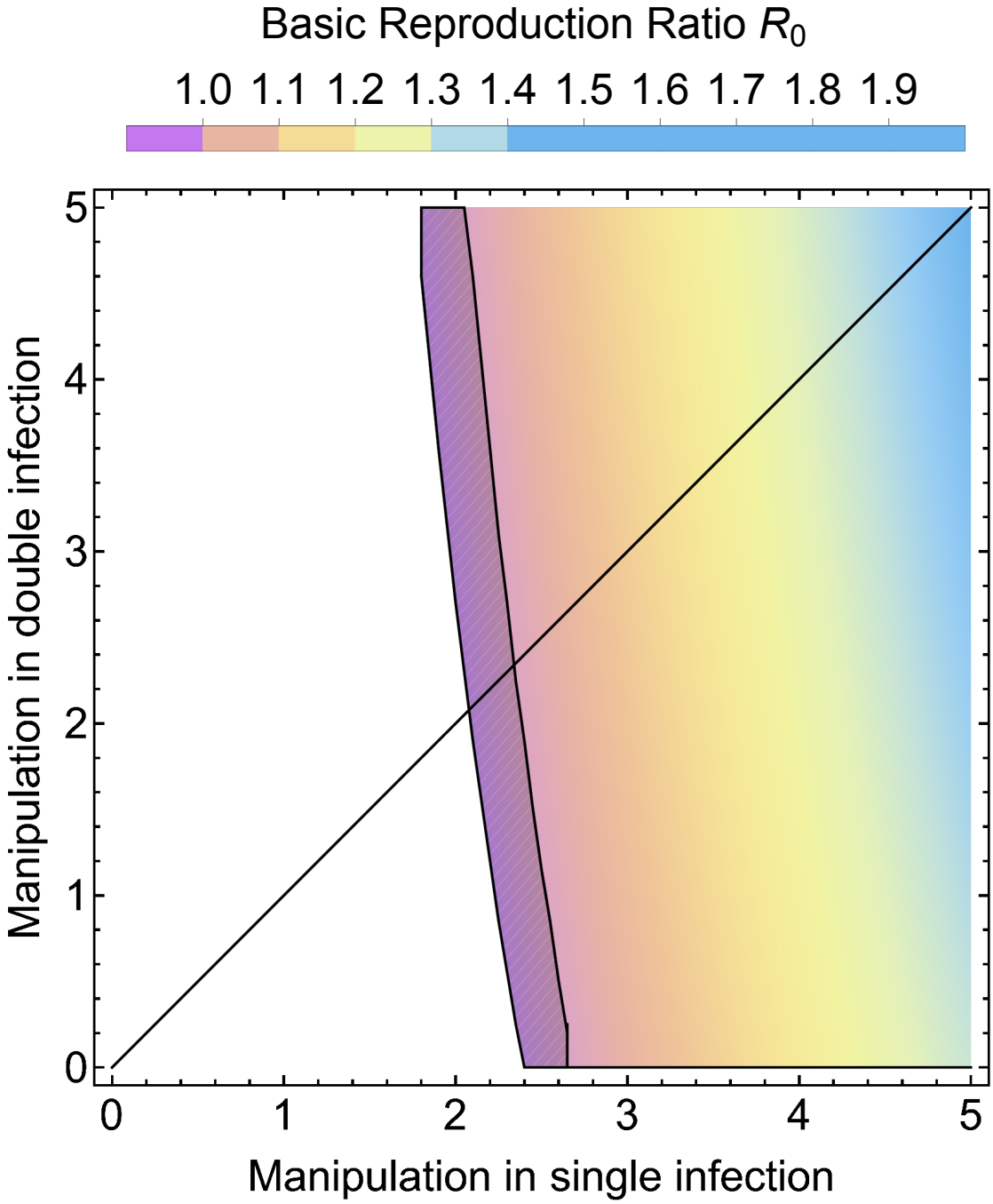

Figure 6: R_0 values increase with more efficient manipulation in both single and double infection. The parasite goes extinct if its manipulative ability is insufficient (~~dotted~~ white area). Bistability region occurs when cooperation in manipulation is intermediate (hatched area). As manipulation in single infection increases, the system only has one stable equilibrium. On the black line, manipulation is indifference between single infection and double infection ($\beta_w = \beta_{ww}$). ~~$R_0 < 1$ in~~ Other parameters are ~~the hatched area indicates that the parasite cannot establish~~ same as in ~~a disease free prey-predator population~~Figure 6. $f_w = 30$

If parasites are uncooperative in both reproduction and manipulation, they can not persist (Figure 7). In contrast, if they are highly cooperative in both aspects (i.e. $f_{ww}/f_w -> \infty$ and $\beta_{ww}/\beta_w -> \infty$), there is one guaranteed single equilibrium for parasite existence.

For intermediate cooperation of both reproduction and manipulation, the bistable area could occur. However, the size of this area is highly sensitive to the value of reproduction and manipulation in single infection. In particular, higher values of these two parameters reduce the bistability area, whereas larger values increase the bistability area (Figure 7~~)~~, Figure SI1). If the parasites sabotage in host manipulation, the system is highly prone to bistability, and only has a single equilibrium when cooperation in reproduction is extremely high. Interestingly, sufficiently high cooperation in reproduction leads to bistability (i.e. f_{ww} is at least 4 times f_w), and noncooperation in reproduction always lead to single equilibrium of the system (Figure 7). While a single equilibrium guarantees existence of parasite population, bistability indicates that a disturbance of the system may likely lead to extinction of parasite. This suggests that whether or not cooperation benefits the population is context dependent. Clearly, if there is no limitation to cooperation, and if reproduction or manipulation in single infection is large enough, cooperation always hold an advantage. On the other hand, cooperation in one aspect or insufficient cooperation in both aspects may make the parasite population more vulnerable to extinction than systems with less cooperative parasites. ~~This suggests that systems in which parasites have much higher manipulative ability and reproduction rate when co-infected than when singly infected are more prone to instability than systems with less cooperative parasites or systems with parasites that sabotage each other in co-infection. In other words, having the best of both worlds, effective manipulation and reproduction, at the individual level may not benefit the population as a whole.~~

~~Increasing the co-transmission probability p~~ Co-transmission probability from the parasite pool to intermediate hosts ~~reduces the extinction area . When p is high, doubly infected intermediate hosts are more abundant. Cooperation in host manipulation then need not be too high to bring the population out of the bi-stability state. However, it also means that the singly infected intermediate hosts are few and parasites in a single infection must make more manipulative effort to successfully transmit (Figure 7B). On the other hand, increasing the~~ has opposite effect on the bistable area compared to co-transmission probability q ~~from intermediate hosts to definitive hosts broadens the extinction area . When~~ from intermediate hosts to intermediate hosts (Figure 8). In particular, when parasite sabotage the manipulation, increasing p enlarge the bistable area whereas increasing q ~~is high, successful transmission to definitive hosts relies on the predation of susceptible definitive hosts on doubly infected intermediate hosts. Cooperation in manipulation, therefore, needs~~

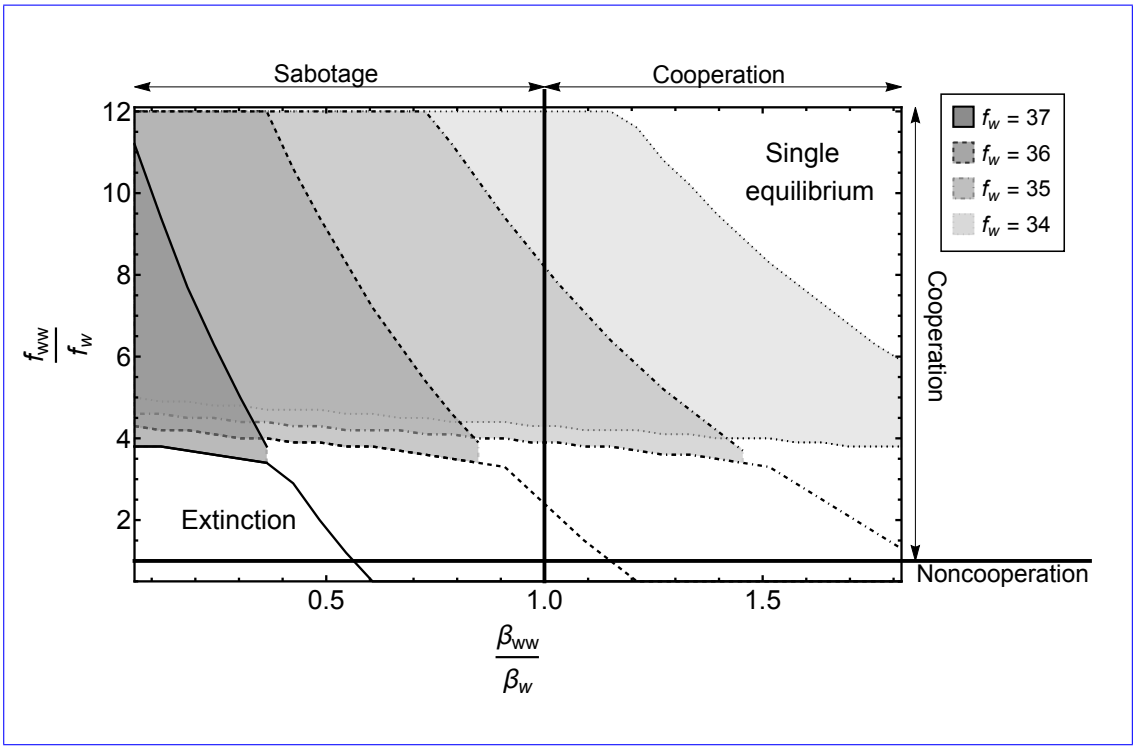

Figure 7: Changes of the bistability area (shaded areas) with respect to different reproduction rates in single ~~and double~~ infection (different boundary styles)~~, and varying cotransmission probability~~. Manipulation and reproduction is indifference between single infection and double infection on the ~~black line~~vertical and horizontal lines respectively. Common parameter: ~~$\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = 0$, $\alpha_{ww} = 0$, $p = 0.1$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = 0$, $\sigma_w w =$ for the thick boundary $\epsilon = 0.5$, $f_w = 36$, the dashed boundary $\epsilon = 1$, $f_w = 36$, and the dot-dashed boundary $\epsilon = 2$, $f_w = 35$.~~

~~to be sufficiently high to avoid bi-stability. Sequential transmission is also rarer because the probability of a single infection $1 - q$ is low. Suppose the number of doubly infected intermediate hosts is low. In that case, general transmission from intermediate hosts to definitive hosts is limited, which explains the wide extinction area~~reduce it. In contrast, when parasites cooperate in manipulation, reducing p decreases the bistable area while reducing q widen it. Evidently, if cooperation in manipulation is extremely high then regardless of the co-transmission value, the population will always exist with one single stable equilibrium. However, as there are always limitation and trade-off in nature, extremely high values may not possible. Considering bistablility indicates vulnerability to disturbance, this suggests that cooperation in manipulation may be beneficial when the co-transmission from the pool to intermediate host increases. However, cooperation in manipulation may be harmful to the

population when the co-transmission from intermediate host to definitive host increases.

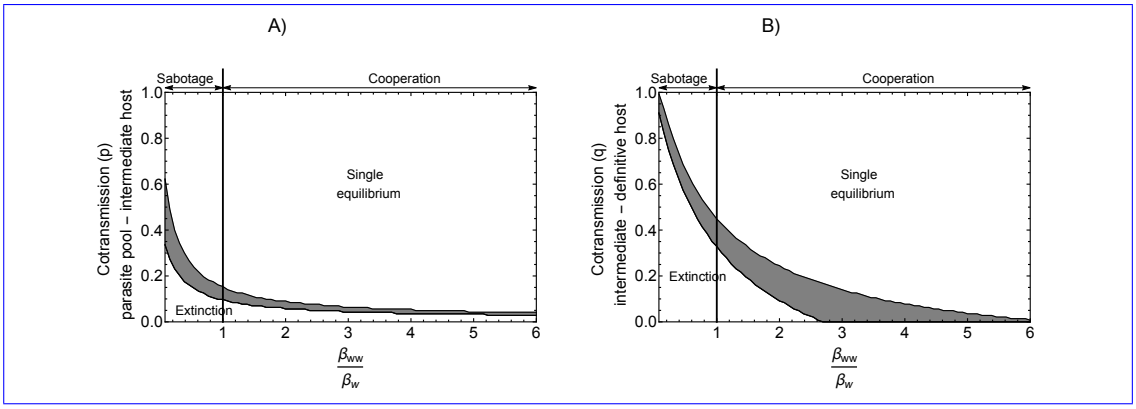

Figure 8: Common parameter: $\rho = 1.2,\ d = 0.9,\ r = 2.5,\ \gamma = 2.9,\ \alpha_w = 0,\ \alpha_{ww} = 0,\ p = 0.05,\ c = 1.4,\ \mu$

Discussion & Conclusion

Host manipulation is a ubiquitous phenomenon suggested to affect the prey-predator dynamics in trophically transmitted parasites. In particular, manipulation of infected intermediate hosts to increase the predation rate of definitive hosts may result in a heavy burden of predators on the intermediate host population. This pressure can make parasites more vulnerable to extinction (Hadeler and Freedman, 1989; Fenton and Rands, 2006).

Our model shows that parasites cannot spread quickly in a cyclic predator-prey system. This delay is an expected result since even though the parasite's basic reproduction ratio R_0 is larger than one, it is estimated at the predator and prey's unstable equilibrium (or cyclic equilibrium). Thus when the density of the prey and predator is at the minimum value of the cycle, the "effective" R_0 of the parasite can be smaller than one. Another interesting result is that the reproduction value is much larger than other parameter values. This result is likely due to the introduction of a free-living parasitic pool. Our model shows that in making the system more realistic, we also obtain a more realistic quantitative value for parasitic reproduction.

In the study by Rogawa et al. (2018), a non-manipulative parasite can invade a susceptible prey-predator population and causes the system to cycle. The system is stabilised when the parasite becomes manipulative, and the stability increases with the manipulative ability. In our model, non-manipulative parasites cannot persist in the system~~. The parasite does not necessarily destabilise the predator-prey system , which may contradict the result of Rogawa et al. (2018)~~, and the parasite never leads the system into cyclic dynamics. These

results may contradict with Rogawa et al. (2018), where non-manipulative parasites lead to a stable system, while increasing manipulation induces the cyclic behaviour of the population. We suggest that the different results may be due to our introduction of a parasite pool and multiple infections, unlike the model of Rogawa et al. (2018). In their system, transmission from the definitive host to the intermediate host was assumed to result from direct contact between the two hosts. Such immediate transmission could directly accelerate the feedback loop between prey and predator. Hence, faster predator-prey dynamics occur, which may lead to cyclic dynamics when parasites are introduced.

~~However, in~~ In our study, ~~host manipulation can destabilise the predator-prey system under particular circumstancesand in a different way than the models of Rogawa et al. (2018). In particular, the destabilisation of the system is possible~~ population dynamics exhibit bistability under certain circumstances. This is very likely due to the ~~occurrence of bistability when parasite reproduction in coinfection is boosted~~introduction of co-transmission, which has been shown to result in bistable population dynamics in plant virus Allen et al. (2019) and infectious disease Gao et al. (2016). In this bistability region, if the system is disturbed (e.g. migration of the intermediate or definitive hosts or predation of intermediate hosts by other predators), then the density of the infected hosts may crash, leading to parasite extinction. In other word, it is a way of destabilising the predator-prey system that is different from the result of Rogawa et al. (2018) (where destabilising means cyclic behaviour). In particular, the destabilisation of the system is possible due to the occurrence of bistability when parasite reproduction in coinfection is boosted. The bistability region widens as ~~the manipulation in double infection increases, and manipulation in a single infection is insufficient~~parasites cooperate in reproduction but sabotage in manipulation. This extension is because the density of the doubly infected hosts is always much smaller than the singly infected host density, limited by sequential transmission and a small probability of co-transmission. Suppose manipulation in a single infection is not sufficient. In that case, the transmission of the parasites depends mainly on the double infection hosts, which is rare. So extinction is possible if manipulation in double infection is not sufficiently high.

Iritani and Sato (2018) show that manipulative parasites can persist if they can alternate manipulation between enhancing and suppressing predation rate. In our model, the parasite cannot switch its manipulative strategy. However, we show that sabotage in manipulation when parasites are coinfected ~~almost~~ always leads to a single stable equilibrium scenario when there is no cooperation in reproduction. This result suggests that manipulation suppression, either by alternating manipulative strategy or sabotaging, can be crucial in maintaining the parasite population.

Finally, our study focuses on the ecological dynamics of the trophically transmitted para-

site. However, investigating the evolution of host manipulation is a natural extension beyond the scope of a single manuscript, given the complexities that arise in the ecological dynamics itself~~(we tried). The~~ . Studying evolution of host manipulation taking into account free-living parasite pool calls for thorough analyses, which itself could be a standalone study. In addition, the occurrence of bistability in our model suggests that the evolution of host manipulation may drive the parasite population to extinction simply because of the scarcity of the mutant and the Allee effect in the population dynamics. ~~Moreover, the~~ The parasite can enhance both values if there is no tradeoff between manipulation and reproduction. Nevertheless, our model shows that this strategy, which seems to make the best of both worlds, can make the system even more unstable. Evolutionary dynamics here depend mainly on the tradeoff between host manipulation and other traits of the parasites, such as reproduction, virulence, and survivorship in the parasite pool, to list a few. This extension deserves thorough analysis, and we will treat it as a separate matter.

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Statement of Authorship

Removed for review

Data and Code Availability

All data and simulation codes for generating figures are available on `https://anonymous.4open.science/r/multipleinfections`

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Tables

~~Figure legends~~

Table 1: Description of variables and parameters

Parameters and Variables	Description
I_i	Density of intermediate hosts that are susceptible $i = s$, singly infected $i = w$, or doubly infected $i = ww$
D_i	Density of definitive hosts that are susceptible $i = s$, singly infected $i = w$, or doubly infected $i = ww$
W	Density of parasites released from definitive hosts into the environment
d	Natural death rate of intermediate hosts
α_i	Additional death rate of intermediate hosts due to infection by a single parasite ($i = w$) or two parasites ($i = ww$)
p	Probability that two parasites cotransmit from the environment to an intermediate host
γ	Transmission rate of parasites in the environment to intermediate hosts
μ	Natural death rate of definitive hosts
σ_i	Additional death rate of definitive hosts due to infection by a single parasite ($i = w$) or two parasites ($i = ww$)
σ_i	Additional death rate of the hosts due to being infected by a singly parasite ($i = w$) or two parasites ($i = ww$)
q	Probability that two parasites cotransmit from intermediate hosts to definitive hosts
β_i	Transmission rate of parasites from intermediate hosts to definitive hosts
f_i	Reproduction rate of parasites in singly infected definitive hosts ($i = w$) or doubly infected hosts ($i = ww$)
δ	Natural death rate of parasites in the environment
h	Probability that the parasites successfully established inside the definitive host