

# On multiple infections by parasites with complex life cycles

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 coinfecting 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, sabotage in manipulation can induce bistability such that a slight disturbance in the system drives the parasite population to extinction. Intriguingly, cooperation in both aspects, host manipulation and reproduction, might ensure system stability. In some cases, a lack of “reproduction cooperation” may 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. Hader 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

59 at the same noninfectious stage, i.e. both parasites are not ready to transmit. Thus the  
60 reduction in mobility is suggested to reduce the predation rate by the definitive hosts. When  
61 two infectious parasites infect the copepods, the copepods' activity increases, and so does the  
62 predation risk for the copepod. However, when the copepods are infected by one infectious  
63 and one noninfectious parasite, their interests clash, and one parasite wins over the other.



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.

64 Theoretical work that considers multiple infections often focuses on the evolution of vir-  
65 ulence (van Baalen and Sabelis, 1995; Alizon et al., 2013; Alizon and van Baalen, 2008;  
66 Choisy and de Roode, 2010; Alizon, 2012). They show multiple infections can increase vir-  
67 ulence (van Baalen and Sabelis, 1995; Choisy and de Roode, 2010). Evolutionary branching

68 of a less virulent and a hypervirulent parasite can occur when considering within-host dy-  
69 namics (Alizon and van Baalen, 2008), and a reduction in virulence is possible if parasites  
70 are co-transmitted (Alizon, 2012). These studies also involve host manipulation to a certain  
71 extent, as it can affect transmission rates, even though they do not explicitly consider the  
72 trait. Host manipulation in trophically transmitted parasites receives less attention. Although  
73 manipulation correlates with the transmission rate in trophically transmitted parasites and  
74 infectious diseases, there are differences. Host manipulation influences the predation rate in  
75 trophically transmitted parasites, predominantly affecting predator-prey dynamics. Theoreti-  
76 cal studies on host manipulation in trophically transmitted parasites with multiple infections  
77 are rare (Parker et al., 2003; Vickery and Poulin, 2009). Moreover, they do not consider the  
78 prey-predator dynamics, which will likely have important feedback on the evolution of host  
79 manipulation. A few studies considering the prey-predator dynamics do not incorporate mul-  
80 tiple infections (Rogawa et al., 2018; Iritani and Sato, 2018; Haderler and Freedman, 1989;  
81 Fenton and Rands, 2006). More importantly, they assume that transmission from definitive  
82 hosts to intermediate hosts is due to direct contact between the two types of hosts. This  
83 is often not the case, as parasites are released from the definitive hosts into the environ-  
84 ment. Transmission happens only when intermediate hosts have contact with this free-living  
85 parasite pool.

86 Our study addresses the gap in the theoretical work on host manipulation in trophically  
87 transmitted parasites. We include multiple infections and consider the dynamics of the free-  
88 living parasite pool. Our compartment model helps illustrate a parasite's complex lifecycle  
89 with two hosts: an intermediate host preyed upon by a definitive host. Transmission from the  
90 intermediate host to the definitive host occurs when predation on infected intermediate hosts  
91 happens. Reproduction only happens in the definitive hosts. New parasites then enter the  
92 environment, where the cycle continues. We focus on the intermediate host manipulation,  
93 such that the parasite increases the uptake of the intermediate host by the definitive host  
94 to increase its transmission rate. We then analyse the effect of host manipulation on the  
95 ecological dynamics in the prey-predator-parasite system. In contrast to the abovementioned  
96 examples, our model consists of a single intermediate host as it already provides enough  
97 complexity to discuss between transmission and manipulation. We found that sabotage in  
98 host manipulation almost always pushes the dynamical system toward bistability, provided the  
99 reproduction in a single infection is sufficiently small. The bistable nature suggests that the  
100 predator-prey parasite system is finely balanced and susceptible to extinction via ecological  
101 disturbances. Initially surprising, we showed that cooperation in both host manipulation and  
102 synergistic reproduction is not always beneficial and might expose the parasite population to  
103 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  $\beta_w$ . However, if the intermediate host is co-infected, the manipulation strategy is  $\beta_{ww}$ . In the scope of this model, we assume no specific relationship between  $\beta_w$  and  $\beta_{ww}$  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,

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

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  $\lambda_w = h_1(\rho + \beta_w)I_w$  and  $\lambda_{ww} = h_2(\rho + \beta_{ww})I_{ww}$ , where  $\rho$  is the baseline 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,

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

123 Definitions of different parameters can be found in Table 1.

124 Here, we focus on manipulation that enhances transmission from intermediate hosts to  
125 definitive hosts; we thus simplify the transmission from the parasite pool to intermediate  
126 hosts such that no sequential infection occurs at this transmission state. This assumption  
127 is motivated given that the prey' lifecycle is often shorter than that of the predator [citation](#).  
128 A prey likely encounters the free-living parasite pool once and then dies due to predation,  
129 making sequential transmission less likely at this state. Sequential infection can happen when  
130 parasites transmit from intermediate hosts to definitive hosts. Therefore, a singly infected  
131 definitive host can be further infected by another parasite if it consumes infected intermediate  
132 hosts. Figure (2) illustrates the system's dynamics.

### 133 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](#),

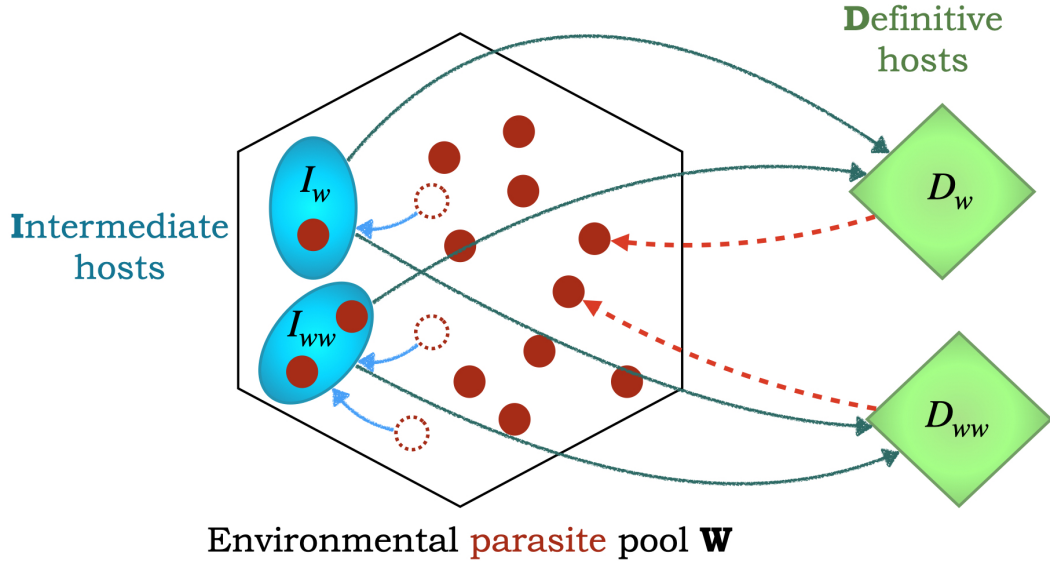


Figure 2: Schematic of the model. Blue ovals represent intermediate host compartment, green diamonds represent definitive host compartment, and the transparent hexagon represents the parasite pool compartment with red circles illustrating individual parasites.

2009; Hurford et al., 2010) (See SI1 for details).

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

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

143 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,

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

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

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

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

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

155 The explicit form of  $I_s^*$  and  $D_s^*$ , capturing the predator-prey dynamics, depends on the  
156 precise form of all birth and predation functions  $B, R, P_s, P_w$  and  $P_{ww}$ . But, it does not  
157 depend on the manipulation ability or any other parameter of the parasite. Given that the  
158 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.

## Birth function of intermediate hosts

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 using the basic reproduction ratio, which is evaluated when the disease-free state is stable. Here,  $R_0 > 1$  happens when  $\gamma$ , 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 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. 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.

The logistic growth for the non-linear birth function 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

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

This equilibrium is 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).

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,  $\alpha_w = \alpha_{ww}$ ,

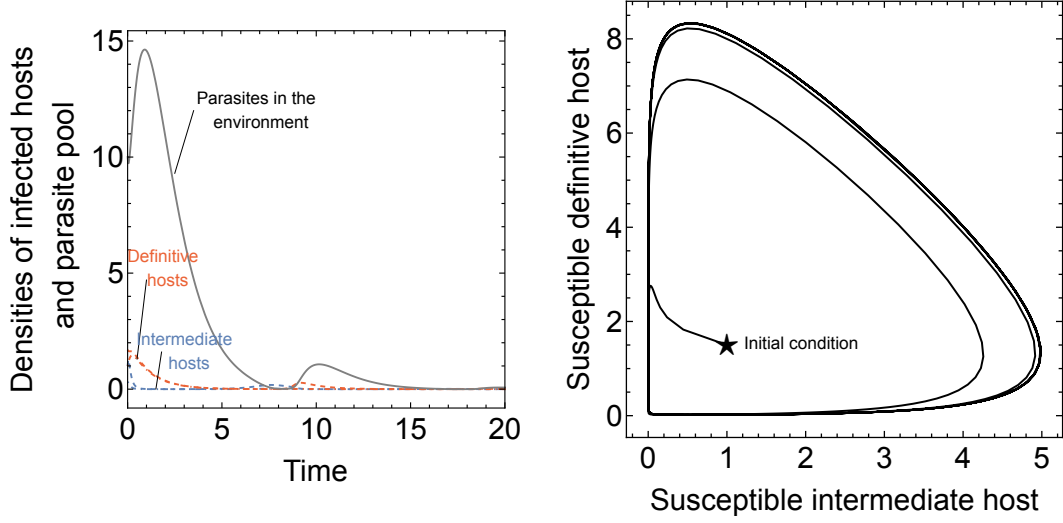


Figure 3: Disease-free equilibrium using linear birth function, where parasite goes extinct (left panel), and susceptible hosts demonstrate cyclic dynamics (right panel). Solid gray line indicate the density of free-living parasites, blue lines indicate infected intermediate hosts while red lines indicate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

186  $\sigma_w = \sigma_{ww}$ , and reproduction in double infection as a linear function concerning reproduction  
 187 in single infections,  $f_{ww} = \epsilon f_w$ . When  $\epsilon > 1$ , reproduction in double infections is greater  
 188 than reproduction in a single infection, whereas  $\epsilon \leq 1$ , reproduction in double infections is  
 189 lower or equal to reproduction in a single infection. We found that the parasite can establish  
 190 if its reproduction value in a single infection  $f_w$  is more significant than a threshold (Figure  
 191 5, see SI5).

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

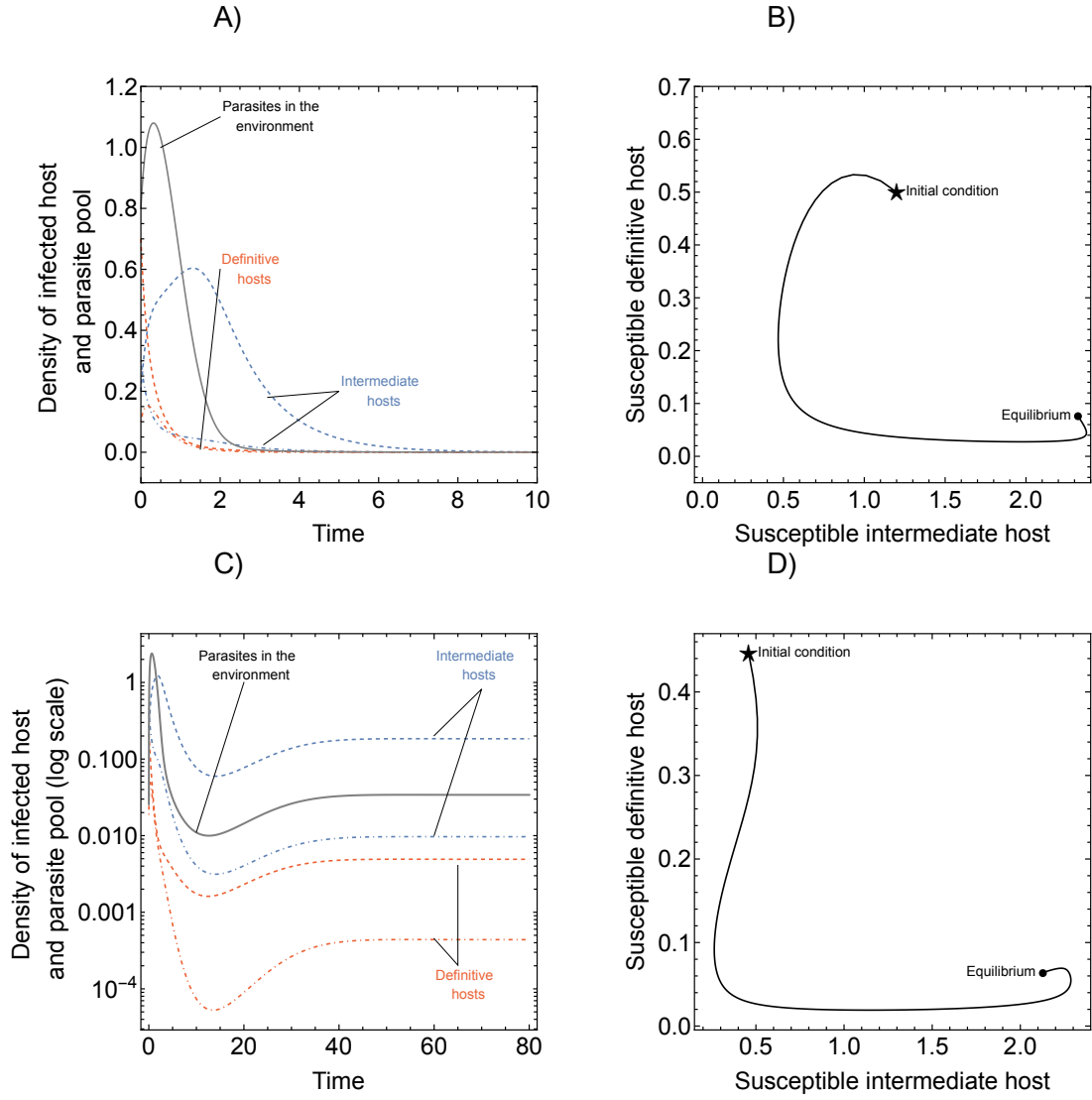


Figure 4: A, B) Disease free equilibrium where parasite cannot persist. C, D) Disease stable equilibrium. Annotations are the same in figure 3. Parameters for disease free equilibrium  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = \alpha_{ww} = 0$ ,  $\beta_w = \beta_{ww} = 1.5$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = \sigma_{ww} = 0$ ,  $q = 0.05$ ,  $f_w = f_{ww} = 7.5$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $h_1 = h_2 = 0.8$ . Disease stable equilibrium have the same parameter values except for higher host manipulation  $\beta_w = \beta_{ww} = 4.5$  and parasite reproduction  $f_w = f_{ww} = 45$

## 199 The effect of host manipulation on ecological dynamics

200 Host manipulation can be cooperative; two parasites increase the predation rate on inter-  
 201 mediate hosts, or  $\beta_{ww} > \beta_w$ . However, it can also be uncooperative; the predation rate on

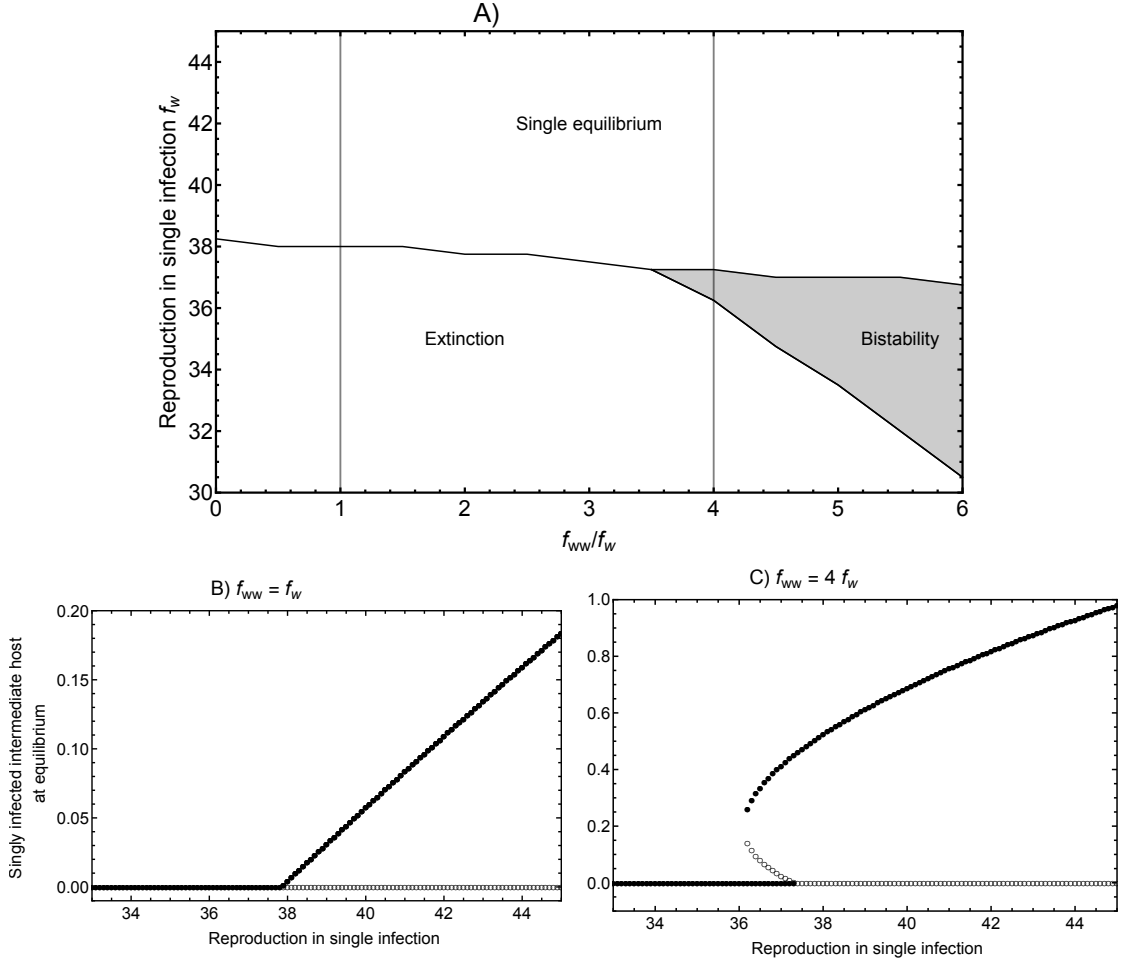


Figure 5: Effect of parasite reproduction on the ecological dynamics. A) Increasing reproduction in doubly infection leads to bistability, B, C) Density of singly infected host at equilibrium when reproduction of parasites are the same in singly and doubly infected hosts  $f_{ww} = f_w$ , and when reproduction of parasites in doubly infected hosts is four times greater than those in singly infected hosts  $f_{ww} = 4f_w$ . Filled circles indicate stable equilibrium and open circles indicate unstable equilibrium. Parameter  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $\beta_w = 1.5$ ,  $\beta_{ww} = 1.5$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $h_1 = h_2 = 0.6$

202 doubly-infected intermediate hosts is lower than that on singly-infected ones or  $\beta_{ww} < \beta_w$ .  
 203 Cooperation in parasite manipulation increases the parasite's basic reproduction ratio  $R_0$ ,  
 204 but the manipulation in a single infection substantially affects the value of  $R_0$  (Figure 6).  
 205 Intuitively, if the manipulation in a single infection is minor, there is not enough transmission,  
 206 and the parasite goes extinct. However, suppose the ability to manipulate the host in a single

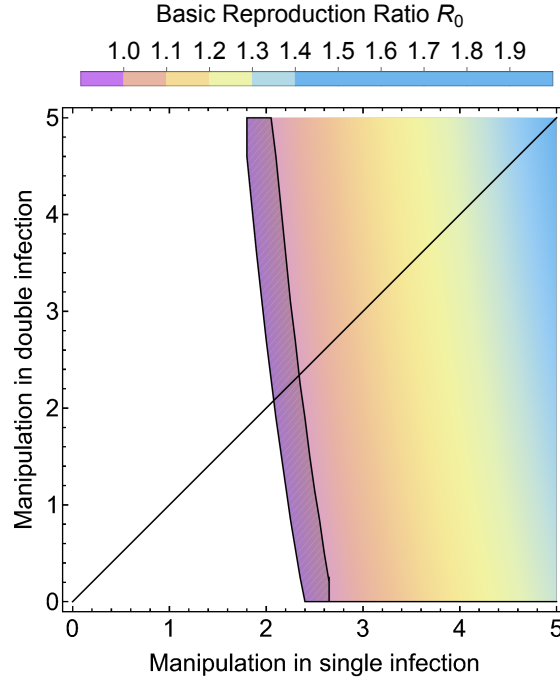


Figure 6:  $R_0$  values increase with more efficient manipulation in both single and double infection. The parasite goes extinct if its manipulative ability is insufficient (white area). Bistability region occurs when cooperation in manipulation is intermediate (hatched area). As manipulation in single infection increases, the system only has one stable equilibrium. On the black line, manipulation is indifference between single infection and double infection ( $\beta_w = \beta_{ww}$ ). Other parameters are the same as in Figure 6.  $f_w = 30$

infection is merely enough for the parasite population to escape extinction. In that case, cooperation in host manipulation leads to a bistable system state. Within the bistable region, the basic reproduction ratio can be less than one, suggesting that the parasite cannot spread when its manipulative values are within this area of weak manipulation when coinfecting.

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 ability and reproduction, we explore all possible combinations of cooperation and sabotage in both manipulation and reproduction. If parasites are uncooperative in reproduction and manipulation, they can not persist (Figure 7). In contrast, if they are highly cooperative in both aspects (i.e.  $f_{ww}/f_w \rightarrow \infty$  and  $\beta_{ww}/\beta_w \rightarrow \infty$ ), there is one guaranteed single equilibrium for

219 parasite existence.

220 For intermediate cooperation of both reproduction and manipulation, the bistable area  
221 could occur. However, the size of this area is sensitive to the value of reproduction and  
222 manipulation in a single infection. In particular, higher values of these two parameters reduce  
223 the bistability area, whereas larger values increase the bistability area (Figure 7, Figure S11).  
224 If the parasites sabotage each other, the system is highly prone to bistability and only has  
225 a single equilibrium when cooperation in reproduction is exceptionally high. Interestingly,  
226 sufficiently high cooperation in reproduction leads to bistability (i.e.  $f_{ww}$  is at least four times  
227  $f_w$ ), and noncooperation in reproduction always leads to a single equilibrium of the system  
228 (Figure 7). While a single equilibrium guarantees the existence of a parasite population,  
229 bistability indicates that a disturbance of the system may likely lead to the extinction of the  
230 parasite population. This suggests that whether or not cooperation benefits the population  
231 is context-dependent. Cooperation always holds an advantage if there are no significant  
232 tradeoffs and if reproduction or manipulation in a single infection is large enough. On the  
233 other hand, cooperation in one aspect or insufficient cooperation in both aspects may make  
234 the parasite population more vulnerable to extinction than systems with less cooperative  
235 parasites.

236 Co-transmission probability from the parasite pool to intermediate hosts  $p$  has the opposite  
237 effect on the bistable area compared to co-transmission probability  $q$  from intermediate hosts  
238 to intermediate hosts (Figure 8). In particular, when the parasite sabotages the manipula-  
239 tion, increasing  $p$  enlarges the bistable area, whereas increasing  $q$  reduces it. In contrast,  
240 when parasites cooperate in manipulation, reducing  $p$  decreases the bistable area while re-  
241 ducing  $q$  widens it. If cooperation in manipulation is exceptionally high, the population will  
242 always exist with one stable equilibrium regardless of the co-transmission value. However,  
243 as there are always limitations and trade-offs, high values may not be possible. Bistability  
244 indicates vulnerability to disturbance, suggesting that cooperation in manipulation may be  
245 beneficial when the co-transmission from the pool to the intermediate host increases. How-  
246 ever, cooperation in manipulation may harm the population when the co-transmission from  
247 the intermediate host to the definitive host increases.

## 248 Discussion & Conclusion

249 Host manipulation is a ubiquitous phenomenon suggested to affect the prey-predator dynam-  
250 ics in trophically transmitted parasites. In particular, manipulation of infected intermediate  
251 hosts to increase the predation rate of definitive hosts may result in a heavy burden of preda-  
252 tors on the intermediate host population. This pressure can make parasites more vulnerable

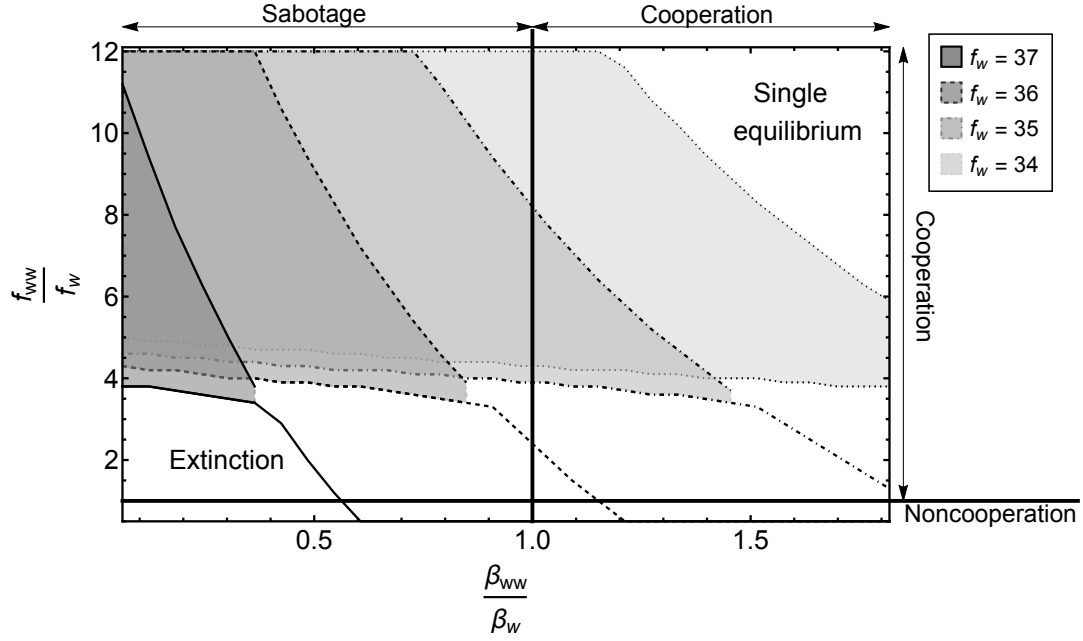


Figure 7: Changes of the bistability area (shaded areas) with respect to different reproduction rates in single infection (different boundary styles). Manipulation and reproduction is indifference between single infection and double infection on the vertical and horizontal lines respectively. Common parameter:  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $\beta_w = 1.65$ ,  $h_1 = h_2 = 0.6$ .

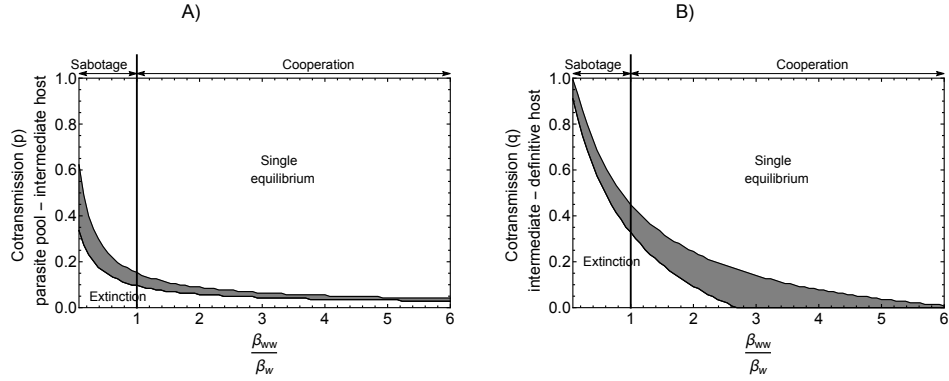


Figure 8: Common parameter:  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $\epsilon = 4.5$ ,  $\beta_w = 1.3$ ,  $f_w = 35$ ,  $h_1 = h_2 = 0.6$ .

253 to extinction (Haderler and Freedman, 1989; Fenton and Rands, 2006).

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

263 In the study by Rogawa et al. (2018), a non-manipulative parasite can invade a susceptible  
264 prey-predator population and cause the system to cycle. We should talk what we mean by  
265 stable and destabilised here The system is stabilised when the parasite becomes manipulative,  
266 and the stability increases with the manipulative ability. In our model, non-manipulative  
267 parasites cannot persist in the system, and the parasite never leads the system into cyclic  
268 dynamics. These results may contradict with Rogawa et al. (2018), where non-manipulative  
269 parasites lead to a stable system while increasing manipulation induces the cyclic behaviour  
270 of the population. We suggest that the different results may be due to our introduction  
271 of a parasite pool and multiple infections, unlike the model of Rogawa et al. (2018). In  
272 their system, transmission from the definitive host to the intermediate host was assumed  
273 to result from direct contact between the two hosts. Such immediate transmission could  
274 directly accelerate the feedback loop between prey and predator. Hence, faster predator-prey  
275 dynamics occur, which may lead to cyclic dynamics when parasites are introduced.

276 In our study, population dynamics exhibit bistability under certain circumstances. This  
277 is very likely due to the introduction of co-transmission, which has been shown to result in  
278 bistable population dynamics in plant virus Allen et al. (2019) and infectious disease Gao  
279 et al. (2016). In this bistability region, if the system is disturbed (e.g. migration of the  
280 intermediate or definitive hosts or predation of intermediate hosts by other predators), then  
281 the density of the infected hosts may crash, leading to parasite extinction. In other words,  
282 it is a way of destabilising the predator-prey system, different from the result of Rogawa  
283 et al. (2018) (where destabilising means cyclic behaviour). In particular, the destabilisation  
284 of the system is possible due to the occurrence of bistability when parasite reproduction in  
285 coinfection is boosted. The bistability region widens as parasites cooperate in reproduction  
286 but sabotage in manipulation. This extension is because the density of the doubly infected  
287 hosts is always much smaller than the singly infected host density, limited by sequential  
288 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 coinfecting 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 parasite. 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. Studying the evolution of host manipulation, considering the free-living parasite pool, calls for thorough analyses, which 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. 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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## 405 Tables

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
$\alpha_i$	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
$\gamma$	Transmission rate of parasites in the environment to intermediate hosts
$\mu$	Natural death rate of definitive hosts
$\sigma_i$	Additional death rate of definitive hosts due to infection by a single parasite ( $i = w$ ) or two parasites ( $i = ww$ )
$\sigma_i$	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
$\beta_i$	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 ( $i = ww$ )
$\delta$	Natural death rate of parasites in the environment
$h$	Probability that the parasites successfully established inside the definitive host