

On multiple infections by parasites with complex life cycles

Phuong L. Nguyen [†] and Chaitanya S. Gokhale [‡]

[†] Department of Biology, University of Fribourg,

Chemin du musée 15, Switzerland

[‡]Max Planck Institute for Evolutionary Biology, Department of Theoretical Biology

August-Thienemann-Straße 2, 24306 Plön, Germany

Center for Computational and Theoretical Biology, University of Würzburg,

Klara-Oppenheimer Weg, 32, 97074, Würzburg, Germany

[†]linh.phuong.nguyen@evobio.eu

[‡]chaitanya.gokhale@uni-wuerzburg.de

1 **Abstract:** Host manipulation is a common strategy of parasites with complex
2 lifecycles. It directly affects predator-prey dynamics in trophically transmitted
3 parasites. Theoretical studies suggest that predation-enhancing manipulation
4 often decimates the prey population, making parasites prone to extinction. Host
5 manipulation, however, can also suppress predation due to conflicting interests
6 when multiple parasites infect a host, often neglected in theoretical studies.
7 Misaligned interests of coinfecting parasites can occur due to limited carrying
8 capacity or parasitoid developmental stage. Including this realistic complexity
9 in a mathematical model, the results depart from previous studies substantially.
10 We show that coinfecting multi-trophic parasites can preserve the predator-prey
11 system and themselves through a combination of manipulation and reproduction
12 parameters. Our study highlights the necessity and provides the means of incor-
13 porating the reality of multiple parasites and their multi-trophic life cycles in the
14 theory of 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. Haderler 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), while host manipulation in trophically transmitted parasites receives less attention. Even though host manipulation and virulence both correlates with parasite transmission, there are subtle differences, such that virulence implies an additional mortality rate to the natural mortality rate of the infected host, while manipulation lead to immediate death of the intermediate host due to higher predation. Host manipulation therefore not only affects the intermediate host population but the entire predator-prey dynamics. Theoretical studies on host manipulation in trophically transmitted parasites rarely consider multiple infections and those that did incorporate this feature neglect the prey-predator dynamics, which will likely have important feedback on the evolution of host manipulation (Parker et al., 2003; Vickery and Poulin, 2009). Moreover, these models assume that transmission from definitive hosts to intermediate hosts is due to direct contact between the two types of hosts (Rogawa et al., 2018; Iritani and Sato, 2018; Haderler



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.

69 and Freedman, 1989; Fenton and Rands, 2006). This is often not the case in nature, as
 70 parasites are released from the definitive hosts into the environment. Transmission thus
 71 happens only when intermediate hosts have contact with this free-living parasite pool. The
 72 inclusion of this free-living stage could induce profound effect on the dynamics of the whole
 73 predator-prey-parasite system.

74 Our study addresses the gap in the theoretical work on host manipulation in trophically
 75 transmitted parasites. We include multiple infections and consider the dynamics of the free-
 76 living parasite pool. Our compartment model helps illustrate a parasite's complex lifecycle

77 with two hosts: an intermediate host preyed upon by a definitive host. Transmission from the
78 intermediate host to the definitive host occurs when predation on infected intermediate hosts
79 happens. Reproduction only happens in the definitive hosts. New parasites then enter the
80 environment, where the cycle continues. We focus on the intermediate host manipulation,
81 such that the parasite increases the uptake of the intermediate host by the definitive host
82 to increase its transmission rate. We then analyse the effect of host manipulation on the
83 ecological dynamics in the prey-predator-parasite system. In contrast to the abovementioned
84 examples, our model consists of a single intermediate host as it already provides enough
85 complexity to discuss between transmission and manipulation. We found that sabotage in
86 host manipulation almost always pushes the dynamical system toward bistability, provided the
87 reproduction in a single infection is sufficiently small. The bistable nature suggests that the
88 predator-prey parasite system is finely balanced and susceptible to extinction via ecological
89 disturbances. Initially surprising, we showed that cooperation in host manipulation and
90 enhanced reproduction in co-infecting parasites is not always beneficial and might expose
91 the parasite population to the risk of extinction.

92 **Model**

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

99 For simplicity, we assume that hosts can be infected by one (single infection) or, at most,
100 two parasites (double infections). Our model is, therefore, more relevant to the macropara-
101 sitic system. Given that infection occurs, the probability that two parasites from the parasite
102 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.

121 For the definitive hosts we have,

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

122 where $B(D_s, D_w, D_{ww}, I_s, I_w, I_{ww})$ represents the birth rate of definitive hosts. The birth
123 rates depend on the density of both intermediate and definitive hosts, infected or uninfected.
124 The force of infection that corresponds respectively to singly infected intermediate host (I_w)
125 and doubly infected intermediate hosts (I_{ww}) is denoted respectively by $\lambda_w = h(\rho + \beta_w)I_w$
126 and $\lambda_{ww} = h(\rho + \beta_{ww})I_{ww}$, where ρ is the baseline predation rate and h is the probability
127 that the parasite successfully establishes inside the host. If there is no manipulation, that is,
128 $\beta_w = \beta_{ww} = 0$, the parasite is still transmitted via the based line predation. The dynamics
129 of the free-living parasites in the environment are then given by,

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

130 Definitions of different parameters can be found in Table SI.1.

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

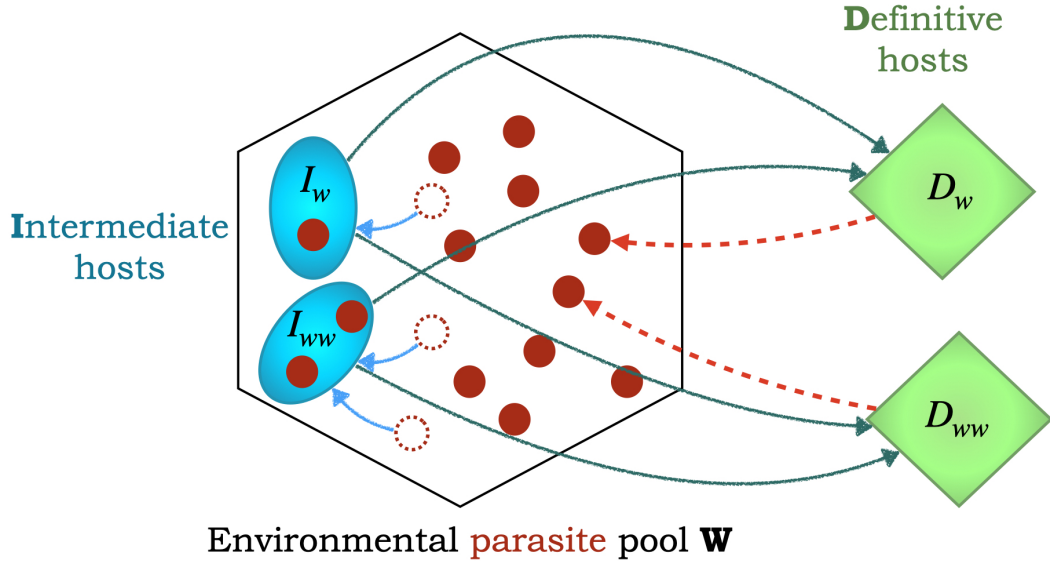


Figure 2: Schematic of the model. Blue ovals represent intermediate host compartment, green diamonds represent definitive host compartment, and the transparent hexagon represents the parasite pool compartment with red circles illustrating individual parasites.

Results

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).

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

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

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

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

168 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 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.

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 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 SI3). However, even when this condition is satisfied, the parasite may not be able to spread and persist in cyclic susceptible host dynamics (Figure SI1). 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 section SI 4. (Figure 3B).

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

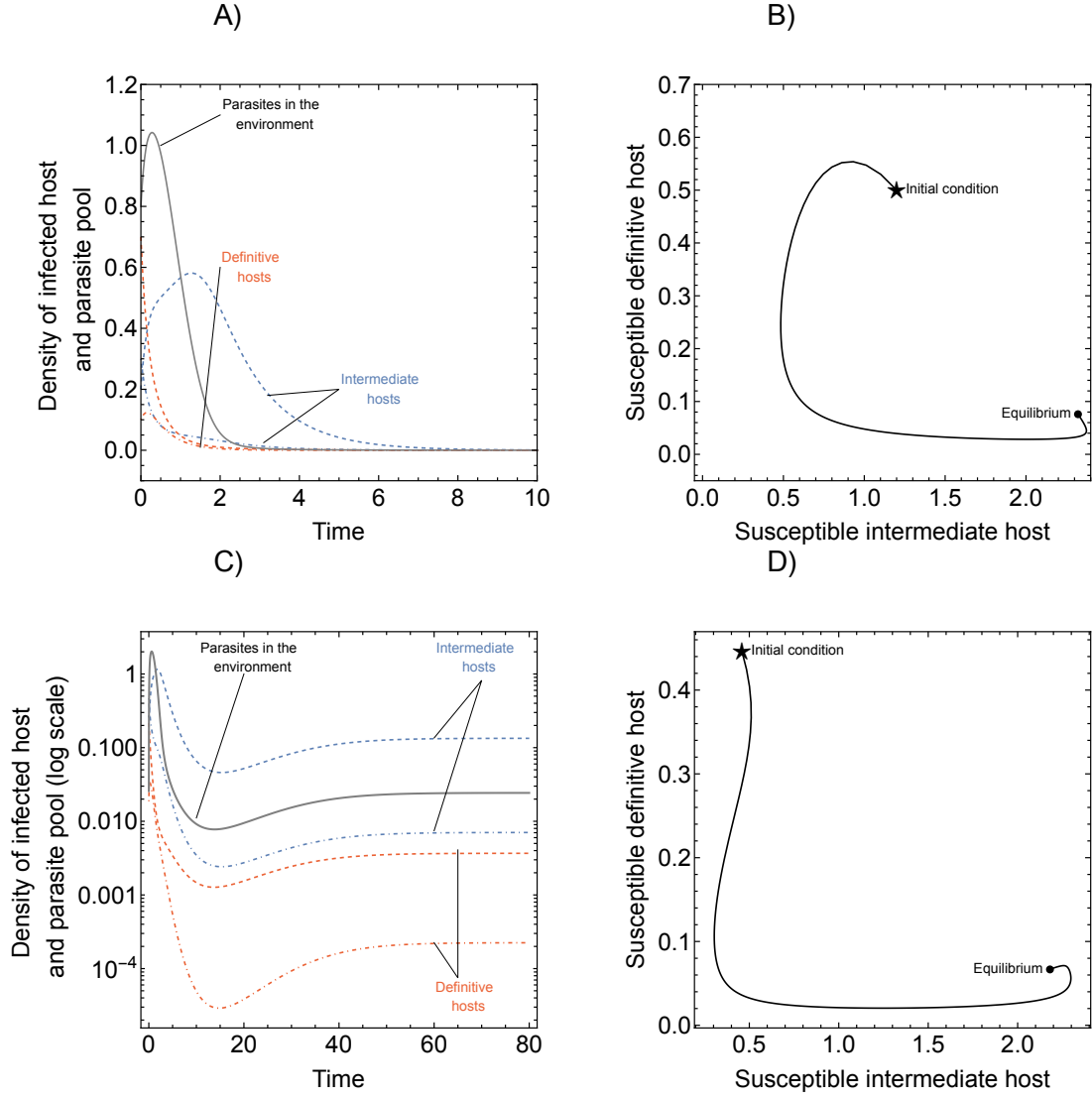


Figure 3: A, B) Disease free equilibrium where parasite cannot persist. C, D) Disease stable equilibrium. Solid gray line indicate the density of free-living parasites, blue lines indicate infected intermediate hosts while red lines indicate infected definitive hosts. Dashed lines indicate singly infected hosts while dot-dashed lines indicate doubly infected hosts. Parameters for disease free equilibrium $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = \alpha_{ww} = 0$, $\beta_w = \beta_{ww} = 1.5$, $p = 0.05$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = \sigma_{ww} = 0$, $q = 0.05$, $f_w = f_{ww} = 7.5$, $\delta = 0.9$, $k = 0.26$, $h = 0.6$. Disease stable equilibrium have the same parameter values except for higher host manipulation $\beta_w = \beta_{ww} = 4.5$ and parasite reproduction $f_w = f_{ww} = 45$

in single infections, $f_{ww} = \epsilon f_w$. When $\epsilon > 1$, reproduction in double infections is enhanced as compared to in single infections, whereas $\epsilon \leq 1$, reproduction in double infections is depressed or equal to reproduction in single infections. We found that the parasite can establish if its reproduction value in a single infection f_w is more significant than a threshold (Figure 4, see section SI 5 and Eq. (SI.19)).

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 enhanced (Figure 4A). 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; the predation rate on doubly-infected intermediate hosts is lower than that on singly-infected ones or $\beta_{ww} < \beta_w$. Cooperation in parasite manipulation increases the parasite's basic reproduction ratio R_0 , but the manipulation in a single infection substantially affects the value of R_0 (Figure 5 Left). Intuitively, if the manipulation in a single infection is minor, there is not enough transmission, and the parasite goes extinct. However, suppose the ability to manipulate the host in a single 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.

Co-infecting parasites can influence each other in different life history traits besides ma-

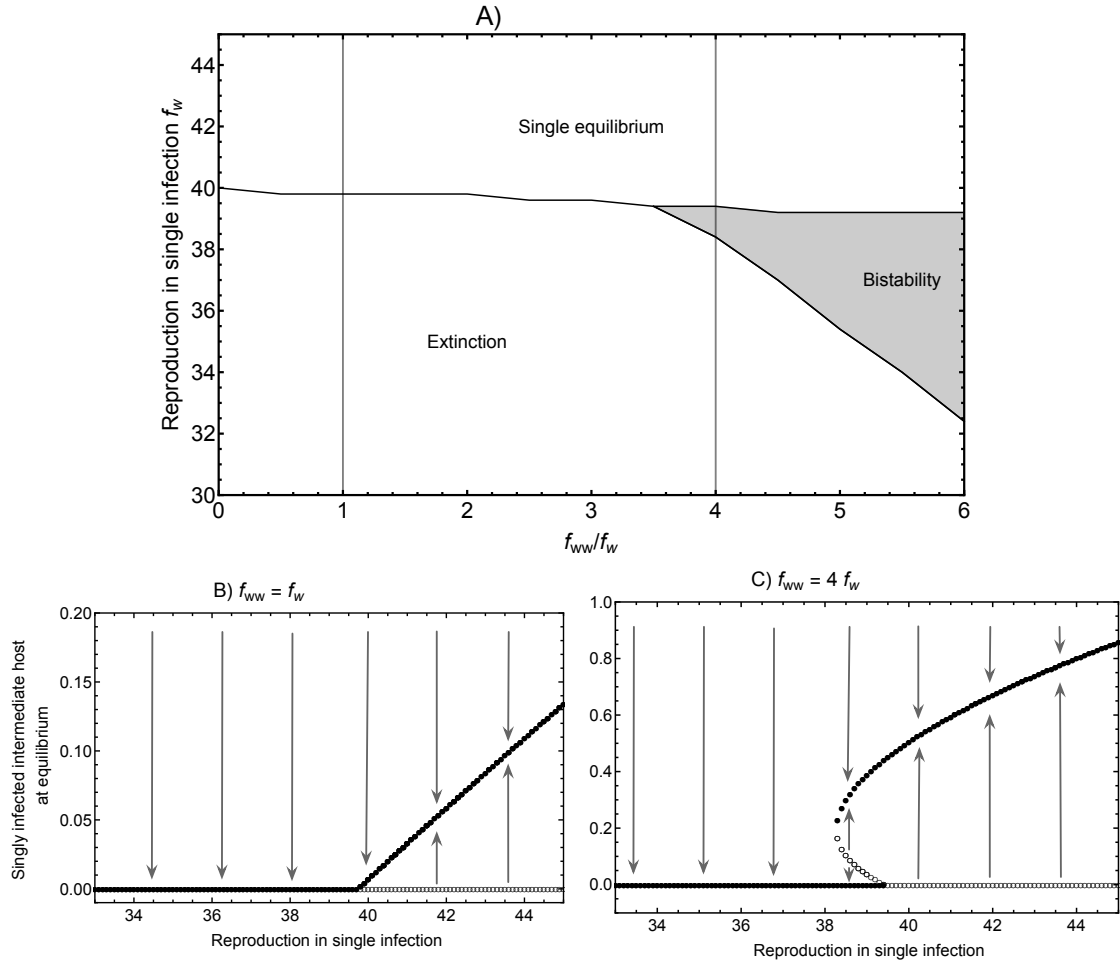


Figure 4: Effect of parasite reproduction on the ecological dynamics. A) Enhanced reproduction in double infection leads to bistability, B, C) Density of singly infected host at equilibrium when reproduction of parasites are the same in singly and doubly infected hosts $f_{ww} = f_w$, and when reproduction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

240 nipulation. Parasites can have an enhanced reproduction rate in coinfections, i.e. $f_{ww} > f_w$.
 241 Likewise, they can compete for resources, so reproduction in double infection is depressed
 242 as compared to in single infection. Without any assumption on the relationship between
 243 manipulative ability and reproduction, we explore all possible combinations of cooperation-

244 sabotage range in manipulation and depressed-enhanced range in reproduction. If parasites
 245 are uncooperative in manipulations and shows depressed reproduction, they cannot persist
 246 (Figure 5). In contrast, if they are highly cooperative in manipulation and show enhanced re-
 247 production (i.e. $\beta_{ww}/\beta_w \rightarrow \infty$ and $f_{ww}/f_w \rightarrow \infty$), there is a guaranteed single equilibrium
 248 for parasite existence.

249 For intermediate levels of coordination in reproduction and manipulation, a bistable area
 250 could occur. However, the size of this area is sensitive to the value of reproduction and
 251 manipulation in a single infection. In particular, higher values of these two parameters
 252 reduce the bistability area, whereas larger values increase the bistability area (Figure 5,
 253 Figure SI.1). If the parasites sabotage each other, the system is highly prone to bistability
 254 and only has a single equilibrium when reproduction is especially enhanced. Interestingly,
 255 sufficiently high reproduction enhancement leads to bistability (i.e. f_{ww} is at least four times
 256 f_w), and depressed reproduction always leads to a single equilibrium of the system (Figure
 257 5). While a single equilibrium guarantees the existence of a parasite population, bistability
 258 indicates that a disturbance of the system may likely lead to the extinction of the parasite
 259 population. This suggests that the benefits of coordination in reproduction and manipulation
 260 are context-dependent. Coordinating holds an advantage if there are no significant tradeoffs
 261 and if reproduction or manipulation in single infections are large enough.

262 Co-transmission probability from the parasite pool to intermediate hosts p has the opposite
 263 effect on the bistable area compared to co-transmission probability q from intermediate hosts
 264 to intermediate hosts (Figure 6). In particular, when the parasite sabotages the manipula-
 265 tion, increasing p enlarges the bistable area, whereas increasing q reduces it. In contrast,
 266 when parasites cooperate in manipulation, reducing p decreases the bistable area while re-
 267 ducing q widens it. If cooperation in manipulation is exceptionally high, the population will
 268 always exist with one stable equilibrium regardless of the co-transmission value. However,
 269 as there are always limitations and trade-offs, high values may not be possible. Bistability
 270 indicates vulnerability to disturbance, suggesting that cooperation in manipulation may be

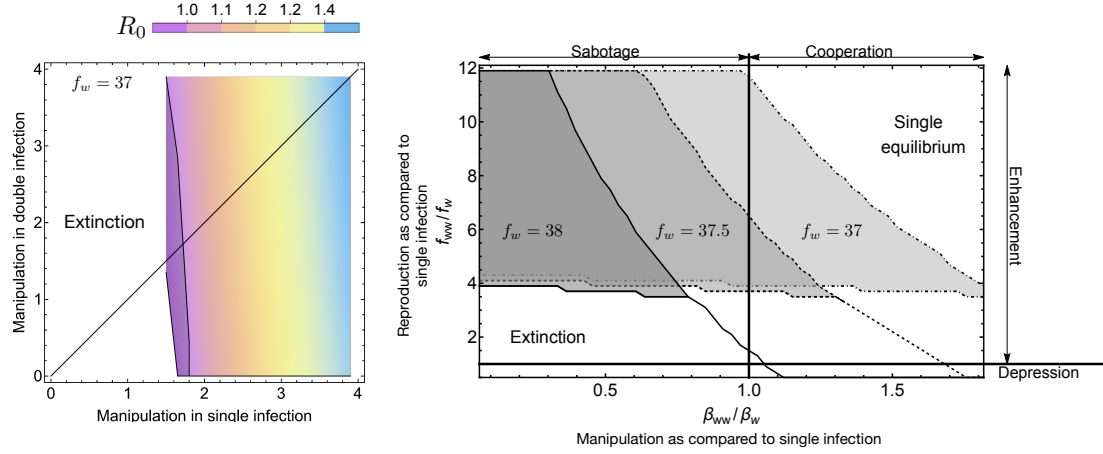


Figure 5: Left: R_0 values increase with more efficient manipulation in both single and double infection. Hatched area indicates bistable region. As manipulation in single infection increases, the system only has one stable equilibrium. On the black line, manipulation is indifference between single infection and double infection ($\beta_w = \beta_{ww}$). Right: 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: $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = 0$, $\alpha_{ww} = 0$, $p = 0.05$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = 0$, $\sigma_{ww} = 0$, $q = 0.05$, $\delta = 0.9$, $k = 0.26$, $\beta_w = 1.65$, $h = 0.6$.

beneficial when the co-transmission from the pool to the intermediate host increases. However, cooperation in manipulation may harm the population when the co-transmission from the intermediate host to the definitive host increases.

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

Our model shows that parasites cannot spread quickly in a cyclic predator-prey system.

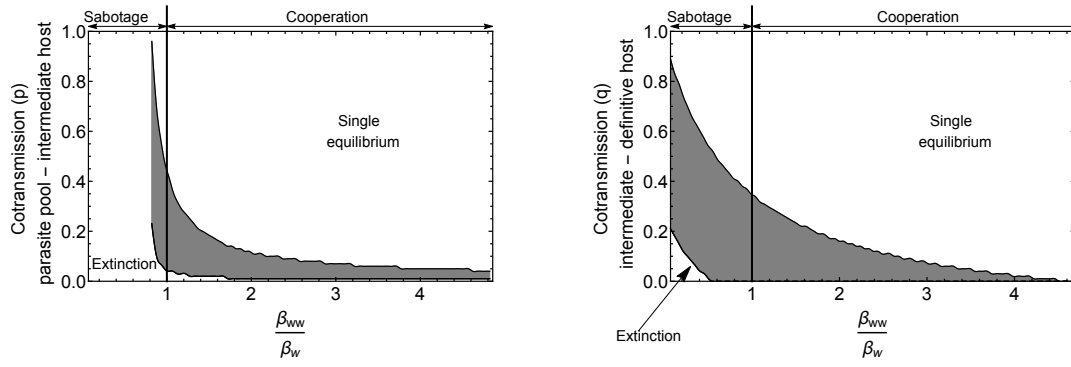


Figure 6: Left: Effect of cotransmission from parasite pool to intermediate host. Right: Effect of cotransmission from intermediate to definitive host. Common parameters: $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = 0$, $\alpha_{ww} = 0$, $p = 0.05$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = 0$, $\sigma_{ww} = 0$, $q = 0.05$, $\delta = 0.9$, $k = 0.26$, $\epsilon = 4.5$, $\beta_w = 1.45$, $f_w = 38$, $h = 0.6$.

281 This delay is an expected result since even though the parasite's basic reproduction ratio R_0
 282 is larger than one, it is estimated at the predator and prey's unstable equilibrium (or cyclic
 283 equilibrium). Thus, when the density of the prey and predator is at the minimum value of the
 284 cycle, the "effective" R_0 of the parasite can be smaller than one. Another interesting result
 285 is that the reproduction value is much larger than other parameter values. This result is
 286 likely due to the introduction of a free-living parasitic pool. Our model shows that in making
 287 the system more realistic, we also obtain a more realistic quantitative value for parasitic
 288 reproduction.

289 In the study by Rogawa et al. (2018), a non-manipulative parasite can invade a susceptible
 290 prey-predator population and cause the system to cycle. The system stops cycling and
 291 approaches a fixed point when the parasite becomes manipulative, and this stability increases
 292 with increased manipulation. In our model, non-manipulative parasites cannot persist in the
 293 system, and the parasite never leads to cyclic dynamics. These results may contradict with
 294 Rogawa et al. (2018), where non-manipulative parasites can still exist via cyclic behaviour.
 295 We suggest that the different results may be due to our introduction of a parasite pool and
 296 multiple infections, unlike the model of Rogawa et al. (2018). In their system, transmission
 297 from the definitive host to the intermediate host was assumed to result from direct contact

298 between the two hosts. Such immediate transmission could directly accelerate the feedback
299 loop between prey and predator. Hence, faster predator-prey dynamics occur, which may
300 lead to cyclic dynamics when parasites are introduced.

301 In our study, population dynamics exhibit bistability under certain circumstances. This
302 is very likely due to the introduction of co-transmission, which has been shown to result in
303 bistable population dynamics in plant virus [Allen et al. \(2019\)](#) and infectious diseases [Gao](#)
304 [et al. \(2016\)](#). In this bistability region, if the system is disturbed (e.g. migration of the
305 intermediate or definitive hosts or predation of intermediate hosts by other predators), then
306 the density of the infected hosts may crash, leading to parasite extinction. The bistability
307 region widens as parasites show enhanced reproduction but sabotage manipulation. This
308 extension is because the density of the doubly infected hosts is always much smaller than
309 the singly infected hosts, limited by sequential transmission and a small probability of co-
310 transmission. If manipulation in a single infection is not sufficient then the transmission of
311 the parasites depends mainly on the doubly infected hosts, which is rare. So, extinction is
312 possible if manipulation in double infections is low.

313 [Iritani and Sato \(2018\)](#) show that manipulative parasites persist if they can alternate
314 manipulation between boosting and suppressing predation rate. In our model, the para-
315 site cannot switch its manipulative strategy. Sabotaging manipulation reduces the basic
316 reproduction ration R_0 and makes the system bistable, exposing the parasite to the risk of
317 extinction. This result contrasts with [Iritani and Sato \(2018\)](#) because in our model, sabotage
318 decreases transmissmion rate from intermediate to definitive host, and does not benefit the
319 parasite.

320 Finally, our study focuses on the ecological dynamics of the trophically transmitted para-
321 site. However, investigating the evolution of host manipulation is a natural extension beyond
322 the scope of a single manuscript, given the complexities that arise in the ecological dynamics
323 itself. Studying the evolution of host manipulation, considering the free-living parasite pool,
324 calls for thorough analyses, which could be a standalone study. For example, we would need

325 to include differences between the traits of the multiple parasites and hence the ecological
326 model becomes more complex than presented in this study. The combinatorics and orderings
327 of sequential infections will then become important. In addition, the occurrence of bistabil-
328 ity in our model suggests that the evolution of host manipulation may drive the parasite to
329 extinction simply because of the rarity of the mutant and the Allee effect as per Adaptive
330 dynamics approaches. The coinfecting parasites can increase manipulation and enhance re-
331 production freely if there exist no tradeoffs. Nevertheless, our model shows that the benefits
332 of this strategy are context-dependent, making it suboptimal in certain cases. Evolutionary
333 dynamics would therefore depend on the tradeoff between host manipulation and other traits
334 of the parasites, such as reproduction, virulence, and survivorship in the parasite pool, to list
335 a few. This extension deserves thorough analysis, and we will treat it as a separate matter.

336 References

- 337 Alizon, S., 2012. Parasite co-transmission and the evolutionary epidemiology of virulence.
338 *Evolution* 67:921–933. URL <https://doi.org/10.1111/j.1558-5646.2012.01827.x>.
- 339 Alizon, S. and M. van Baalen, 2008. Multiple infections, immune dynamics, and the evolution
340 of virulence. *The American Naturalist* 172:E150–E168. URL [https://doi.org/10.](https://doi.org/10.1086/590958)
341 [1086/590958](https://doi.org/10.1086/590958).
- 342 Alizon, S., J. C. de Roode, and Y. Michalakis, 2013. Multiple infections and the evolution of
343 virulence. *Ecology Letters* 16:556–567. URL <https://doi.org/10.1111/ele.12076>.
- 344 Allen, L. J. S., V. A. Bokil, N. J. Cuniffe, F. M. Hamelin, F. M. Hilker, and M. J. Jeger, 2019.
345 Modelling Vector Transmission and Epidemiology of Co-Infecting Plant Viruses. *Viruses*
346 11:1153. URL <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6950130/>.
- 347 van Baalen, M. and M. W. Sabelis, 1995. The dynamics of multiple infection and the

348 evolution of virulence. *The American Naturalist* 146:881–910. URL [https://doi.org/](https://doi.org/10.1086/285830)
349 10.1086/285830.

350 Benesh, D. P., 2016. Autonomy and integration in complex parasite life cycles. *Parasitology*
351 143:1824 – 1846.

352 Choisy, M. and J. C. de Roode, 2010. Mixed infections and the evolution of virulence: Effects
353 of resource competition, parasite plasticity, and impaired host immunity. *The American*
354 *Naturalist* 175:E105–E118. URL <https://doi.org/10.1086/651587>.

355 Diekmann, O., J. Heesterbeek, and J. Metz, 1990. On the definition and the computation
356 of the basic reproduction ratio r_0 in models for infectious diseases in heterogeneous
357 populations. *Journal of Mathematical Biology* 28. URL [https://doi.org/10.1007/](https://doi.org/10.1007/bf00178324)
358 bf00178324.

359 Diekmann, O., J. A. P. Heesterbeek, and M. G. Roberts, 2009. The construction of next-
360 generation matrices for compartmental epidemic models. *Journal of The Royal Society*
361 *Interface* 7:873–885. URL <https://doi.org/10.1098/rsif.2009.0386>.

362 Fenton, A. and S. A. Rands, 2006. The impact of parasite manipulation and predator
363 foraging behavior on predator - prey communities. *Ecology* 87:2832–2841. URL [https://doi.org/10.1890/0012-9658\(2006\)87\[2832:tiopma\]2.0.co;2](https://doi.org/10.1890/0012-9658(2006)87[2832:tiopma]2.0.co;2).

365 Gandon, S., 2018. Evolution and manipulation of vector host choice. *The American Naturalist*
366 192:23–34. URL <https://doi.org/10.1086/697575>.

367 Gao, D., T. C. Porco, and S. Ruan, 2016. Coinfection dynamics of two diseases in a single
368 host population. *Journal of Mathematical Analysis and Applications* 442:171–188. URL
369 <https://www.sciencedirect.com/science/article/pii/S0022247X16300841>.

370 Haderl, K. P. and H. I. Freedman, 1989. Predator-prey populations with parasitic infec-
371 tion. *Journal of Mathematical Biology* 27:609–631. URL [https://doi.org/10.1007/](https://doi.org/10.1007/bf00276947)
372 bf00276947.

373 Hafer, N. and M. Milinski, 2015. When parasites disagree: evidence for parasite-induced
374 sabotage of host manipulation. *Evolution* 69:611 – 620.

375 Hosack, G. R., P. A. Rossignol, and P. van den Driessche, 2008. The control of vector-borne
376 disease epidemics. *Journal of Theoretical Biology* 255:16–25. URL [https://doi.org/](https://doi.org/10.1016/j.jtbi.2008.07.033)
377 10.1016/j.jtbi.2008.07.033.

378 Hughes, D. P., J. Brodeur, and F. Thomas, 2012. *Host Manipulation by Parasites*. Oxford
379 University Press, London, England.

380 Hurford, A., D. Cownden, and T. Day, 2010. Next-generation tools for evolutionary invasion
381 analyses. *Journal of The Royal Society Interface* 7:561–571.

382 Iritani, R. and T. Sato, 2018. Host-manipulation by trophically transmitted parasites: The
383 switcher-paradigm. *Trends in Parasitology* 34:934–944. URL [https://doi.org/10.](https://doi.org/10.1016/j.pt.2018.08.005)
384 1016/j.pt.2018.08.005.

385 Kalbe, M., K. M. Wegner, and T. B. H. Reusch, 2002. Dispersion patterns of parasites in
386 0+ year three-spined sticklebacks: a cross population comparison. *Journal of Fish Biology*
387 60:1529–1542.

388 Lotka, A. J., 1920. Analytical note on certain rhythmic relations in organic systems. *Pro-*
389 *ceedings of the National Academy of Sciences* 6:410–415. URL [https://doi.org/10.](https://doi.org/10.1073/pnas.6.7.410)
390 1073/pnas.6.7.410.

391 Molyneux, D. H. and D. Jefferies, 1986. Feeding behaviour of pathogen-infected vectors.
392 *Parasitology* 92:721–736.

393 Parker, G. A., J. C. Chubb, G. N. Roberts, M. Michaud, and M. Milinski, 2003. Optimal
394 growth strategies of larval helminths in their intermediate hosts. *Journal of Evolutionary*
395 *Biology* 16:47–54. URL <https://doi.org/10.1046/j.1420-9101.2003.00504.x>.

- 396 Ripa, J. and U. Dieckmann, 2013. Mutant invasions and adaptive dynamics in variable
397 environments. *Evolution* 67:1279–1290. URL [https://onlinelibrary.wiley.com/](https://onlinelibrary.wiley.com/doi/abs/10.1111/evo.12046)
398 [doi/abs/10.1111/evo.12046](https://onlinelibrary.wiley.com/doi/abs/10.1111/evo.12046).
- 399 Rogawa, A., S. Ogata, and A. Mougi, 2018. Parasite transmission between trophic levels
400 stabilizes predator–prey interaction. *Scientific Reports* 8. URL [https://doi.org/10.](https://doi.org/10.1038/s41598-018-30818-7)
401 [1038/s41598-018-30818-7](https://doi.org/10.1038/s41598-018-30818-7).
- 402 Rogers, M. E. and P. A. Bates, 2007. Leishmania manipulation of sand fly feeding behavior
403 results in enhanced transmission. *PLoS Pathogens* 3:e91. URL [https://doi.org/10.](https://doi.org/10.1371/journal.ppat.0030091)
404 [1371/journal.ppat.0030091](https://doi.org/10.1371/journal.ppat.0030091).
- 405 Roosien, B. K., R. Gomulkiewicz, L. L. Ingwell, N. A. Bosque-Pérez, D. Rajabaskar, and
406 S. D. Eigenbrode, 2013. Conditional vector preference aids the spread of plant pathogens:
407 Results from a model. *Environmental Entomology* 42:1299–1308. URL [https://doi.](https://doi.org/10.1603/en13062)
408 [org/10.1603/en13062](https://doi.org/10.1603/en13062).
- 409 Seppälä, O. and J. Jokela, 2008. Host manipulation as a parasite transmission strategy
410 when manipulation is exploited by non-host predators. *Biology Letters* 4:663–666. URL
411 <https://doi.org/10.1098/rsbl.2008.0335>.
- 412 Vickery, W. L. and R. Poulin, 2009. The evolution of host manipulation by parasites: a
413 game theory analysis. *Evolutionary Ecology* 24:773–788. URL [https://doi.org/10.](https://doi.org/10.1007/s10682-009-9334-0)
414 [1007/s10682-009-9334-0](https://doi.org/10.1007/s10682-009-9334-0).
- 415 Wedekind, C. and M. Milinski, 1996. Do three-spined sticklebacks avoid consuming cope-
416 pods, the first intermediate host of *Schistocephalus solidus*? - an experimental analysis
417 of behavioural resistance. *Parasitology* 112:371–383. URL [https://doi.org/10.1017/](https://doi.org/10.1017/s0031182000066609)
418 [s0031182000066609](https://doi.org/10.1017/s0031182000066609).
- 419 Zimmer, C., 2001. *Parasite Rex: Inside the Bizarre World of Nature's Most Dangerous*
420 *Creatures*. Atria Books.