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Hyperparasitism and the evolution of parasite virulence

Jason Wood[1,*] and Ben Ashby[1,2,3]

1. Department of Mathematical Sciences, University of Bath.

2. Department of Mathematics, Simon Fraser University.

3. The Pacific Institute on Pathogens, Pandemics and Society (PIPPS), Simon Fraser University, Burnaby, BC

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*Corresponding author: jrw78@bath.ac.uk

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ABSTRACT

Hyperparasites (species which parasitise other parasites) are common in natural populations and affect many parasitic taxa, including: eukaryotic parasites; bacterial and fungal pathogens; and insect parasitoids. Hyperparasitism is therefore likely to shape the ecology and evolution of many host-parasite systems, and represents a promising method for biocontrol (e.g. treating antimicrobial resistant infections). However, the eco-evolutionary consequences of hyperparasitism have received little attention. We use a host-parasite-hyperparasite model to explore how introducing a hyperparasite drives the evolution of parasite virulence, and how this affects host population dynamics. We show when the introduction of a hyperparasite selects for higher or lower parasite virulence, and how this changes the disease burden for the host population. Crucially, we show that variation in the virulence and infectivity of hyperparasites, along with the probability of co-transmission, can lead to a previously unseen hysteresis effect, whereby small shifts in hyperparasite characteristics can lead to sudden shifts in parasite virulence. We show that hyperparasites can induce diversification in parasite virulence, leading to the coexistence of high and low virulence strains. Our results show hyperparasites can have dramatic effects on the evolution of parasite virulence, and that the use of hyperparasites in biocontrol should therefore be approached with caution.

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INTRODUCTION

Hyperparasitism, wherein parasitic organisms are themselves parasitised by another species, is ubiquitous in the natural world. Hyperparasitism has been observed across many taxa, including bacterial (Greer 2005; Ashelford, Day, and Fry 2003) and fungal (Mikhailov, Simdyanov, and Aleoshin 2016) pathogens, parasitic worms (Mohan et al. 2020) and other eukaryotic parasites (Wendling et al. 2017), and among insect parasitoids (Haelewaters, Page, and Pfister 2018). For example, many human bacterial pathogens are parasitised by bacteriophages, including *E. Coli* (e.g. phage O157:H7 (Munns et al. 2015)); *Salmonella typhimurium* (e.g. phage ϕAB2 (Berchieri, Lovell, and Barrow 1991)); and *Klebsiella pneumoniae* (e.g. phage B5055 (Chhibber, Kaur, and Kumari 2008)). Hyperparasitism is also common among fungal plant pathogens, such as the powdery mildew fungus *Podosphaera plantaginis* with the hyperparasitic fungus *Ampelomyces* spp. (Parratt and Laine 2018), or the chestnut blight fungus *Cryphonectria parasitica* (Nuss 2005) which can be infected by *Cryphonecria hypovirus 1* (Parratt and Laine 2016).

Hyperparasites are understood to play a major role in the ecology of parasites, and have been shown to influence both the early epidemic dynamics and the overwintering success (Parratt and Laine 2018) of plant pathogens. They have also been implicated as a driver of seasonal epidemics of cholera (Faruque et al. 2005). In addition to ecological effects on the parasite, hyperparasites can have significant effects on virulence, either inducing hypovirulence (a reduction in the virulence experienced by the host, e.g. by reducing the population size of the parasite) or hypervirulence (an increase in the virulence experienced by the host, e.g. by causing the parasite to release toxins or by introducing virulence factors). Hyperparasite-induced changes in virulence have been observed in many bacterial pathogens. For example, a phage protein enhances motility (and hence virulence) of *E. coli* (Kakkanat et al. 2017); the temperate phage PHB09 reduces the virulence of *Bordetella bronchiseptica* both *in vivo* and *in vitro* (Chen et al. 2020); the phage CTXϕ encodes the cholera toxin

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within *Vibrio cholerae* (Waldor and Mekalanos 1996); and the phage ϕCDHM1 interferes with quorum sensing in *Clostridium difficile* (Hargreaves, Kropinski, and Clokie 2014).

Due to their ecological and virulence-mediating effects, hyperparasites long have been considered as possible sources of biological control (biocontrol) for many infectious diseases (Holtappels et al. 2021; Obradovic et al. 2004), including in the agricultural and food industries and in the treatment of chronic or antimicrobial resistant infections in humans (Gordillo Altamirano and Barr 2019). Indeed, phage therapy has long been used as an alternative to antibiotics in some countries (Schooley et al. 2017; Ferriol-González and Domingo-Calap 2021). However, little is known about the evolutionary implications of hyperparasitism for parasites, and so their use as agents of biocontrol in novel settings could lead to unexpected outcomes for important traits such as parasite infectivity and virulence. For instance, (Prospero et al. 2021) speculated that the introduction of hyperparasites which reduce parasite virulence (hypovirulence) might select for higher virulence in chestnut blight fungus, which has been observed experimentally (Bryner and Rigling 2012).

Understanding the interplay of host, parasite and hyperparasite ecological and evolutionary dynamics is crucial not only to the development of novel agents of biocontrol, but also for understanding their role in natural ecosystems. Theoretical studies of hyperparasites are rare and have in the past mainly focussed on their ecological consequences (Taylor et al. 1998; Morozov, Robin, and Franc 2007). Yet recently there has been renewed theoretical interest in hyperparasitism in an evolutionary context, in the form of theoretical models of hyperparasite evolution (Northrup et al. 2021) and parasite-hyperparasite coevolution (Sandhu et al. 2021). Two findings are of particular interest. First, Sandhu et al. (2021), who considered parasite-hyperparasite coevolution, observed that the introduction of hyperparasites typically increases parasite virulence, but in almost all scenarios decreases average host mortality. Second, Northrup et al. (2021) observed that when

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hyperparasites are more readily co-transmitted with evolutionarily static parasites, this selects for less harm by the hyperparasite due to an increased link between its fitness and parasite transmission (similar to virulence evolution in vertically transmitted parasites). These findings highlight the importance of understanding hyperparasitism in both an ecological and an evolutionary context.

Here, we further investigate the eco-evolutionary consequences of introducing of a hyperparasite for parasite virulence and the resulting net impact on the host population. We show how the introduction of hyperparasites can cause an increase in evolved virulence but may cause an increase or decrease in disease burden depending on the relative infectivity and virulence of hyperparasitised parasites. We also show how small changes in the effects of the hyperparasite on parasite transmission and virulence can lead to large shifts in the evolution of virulence due to hysteresis. Finally, we show that the hyperparasite can induce diversification in parasite virulence, leading to the coexistence of a relatively high and low virulence strains.

METHODS

MODEL DESCRIPTION

We consider a well-mixed population of asexual hosts, parasites, and hyperparasites, where S is the density of uninfected (susceptible) hosts, I is the density of hosts only infected by the parasite (parasitised) and H is the density of hosts infected by both the parasite and the hyperparasite (hyperparasitised). Hosts reproduce at a baseline per-capita rate b, subject to density-dependent crowding qN, with $q > 0$ and $N = S + I + H$. Parasite transmission is density-dependent, with parasitised and hyperparasitised hosts having parasite transmission rates of β and $\eta\beta$ to susceptible hosts, respectively, where β is the baseline transmission rate, $\eta > 1$ implies "hypertransmission" (the hyperparasite increases parasite transmissibility) and $\eta < 1$ implies "hypotransmission" (the

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hyperparasite decreases parasite transmissibility). Hyperparasites are co-transmitted with parasites to susceptible hosts with probability ρ. Hyperparasite infection of parasitised hosts is also density-dependent, with transmission rate σ. All hosts experience a natural mortality rate d, with parasitised and hyperparasitised hosts experiencing additional mortality due to disease at rates α and $\lambda\alpha$, respectively. Thus, when $\lambda < 1$ the hyperparasite induces hypovirulence (the hyperparasite decreases the disease-associated mortality rate) and when $\lambda > 1$ the hyperparasite induces hypervirulence (the hyperparasite increases the disease-associated mortality rate). Both parasitised and hyperparasitised hosts recover from infection at rate γ, with no lasting immunity (see Table 1 for a full summary of model parameters and their default values for the analysis).

The ecological dynamics for a monomorphic population are described by the following three ordinary differential equations:

$$\begin{aligned} \dot{S} &= (b - qN)N - (\beta I + \eta\beta H + d)S + \gamma(I + H) \\ \dot{I} &= \big(\beta S - \sigma H - (d + \alpha + \gamma)\big)I + (1 - \rho)\eta\beta SH \\ \dot{H} &= \big(\rho\eta\beta S + \sigma I - (d + \lambda\alpha + \gamma)\big)H \end{aligned} \qquad (1)$$

Parameter	Description	Default value
b	Natural birth rate of hosts	2.0
d	Natural mortality rate of hosts	0.5
q	Strength of density dependent competition	0.1
α_{max}	Maximum parasite virulence	5.0
β_0	Scale factor in parasite virulence-infectivity trade-off	$\sqrt{5}$
γ	Host recovery rate	0.5
η	Hyperparasite infectivity modifier	n/a

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λ	Hyperparasite virulence modifier	n/a
ρ	Probability of hyperparasite co-transmission	0.5
σ	Hyperparasite transmission rate	4.0

Table 1: Model parameters and default values

We explore the evolution of parasite virulence under a standard transmission-virulence trade-off with diminishing returns, such that $\beta = \beta(\alpha)$ and $\beta''(\alpha) < 0$. For simplicity, we consider a trade-off of the form,

$$\beta(\alpha) = \beta_{max}\sqrt{\frac{\alpha}{\alpha_{max}}} = \beta_0\sqrt{\alpha} \tag{2}$$

We assume that mutations are sufficiently rare so that the ecological dynamics in Equation 1 reach a stable endemic equilibrium (with either the host and parasite, or all three species present; see *Supplementary materials*) before a new mutant arises, and that mutations have small phenotypic effects. The invasion dynamics of a rare mutant parasite with transmission rate β_m and virulence α_m are then,

$$\begin{aligned} \dot{I_m} &= \left(\beta_m S^* - \sigma H^* - (d + \alpha_m + \gamma)\right)I_m + (1-\rho)\eta\beta_m S^* H_m, \\ \dot{H_m} &= \left(\rho\eta\beta_m S^* - (d + \lambda\alpha_m + \gamma)\right)H_m + \sigma I_m H^*. \end{aligned} \tag{3}$$

where asterisks indicate the equilibrium population densities for the resident parasite. The invasion fitness $w(\alpha_m)$ is given by the largest eigenvalue of the next generation matrix (Hurford, Cownden, and Day 2010) (see *Supplementary materials*),

$$\begin{bmatrix} \dfrac{\beta_m S^*}{\sigma H^* + d + \alpha_m + \gamma} + \dfrac{(1-\rho)\eta\sigma\beta_m S^* H^*}{(\sigma H^* + d + \alpha_m + \gamma)(d + \lambda\alpha_m + \gamma)} & \dfrac{(1-\rho)\eta\beta_m S^*}{d + \lambda\alpha + \gamma} \\ \dfrac{\rho\eta\sigma\beta_m S^* H^*}{(\sigma H^* + d + \alpha_m + \gamma)(d + \lambda\alpha_m + \gamma)} & \dfrac{\rho\eta\beta_m S^*}{d + \lambda\alpha + \gamma} \end{bmatrix}$$

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which we omit here for the sake of brevity. We derive the fitness gradient $F(\alpha) = \frac{\partial w}{\partial \alpha_m}\Big|_{\alpha_m=\alpha}$ and hence calculate singular strategies, α^*, that satisfy $F(\alpha^*) = 0$. We determine the evolutionary stability of a singular strategy by the sign of $E(\alpha^*) = \frac{\partial^2 w}{\partial \alpha_m^2}\Big|_{\alpha_m=\alpha=\alpha^*}$ (negative values indicate evolutionary stability), and convergence stability by numerically approximating the derivative $M(\alpha^*) = \frac{\partial^2 w}{\partial \alpha_m \partial \alpha}\Big|_{\alpha_m=\alpha=\alpha^*}$ and checking if the inequality $E(\alpha^*) + M(\alpha^*) < 0$ holds.

Simulations

We complement our numerical analysis with simulations of the evolutionary dynamics of our system, which relax the adaptive dynamics assumptions of continuous traits and separate ecological and evolutionary timescales. To perform our simulations, we first create a discretised trait space for the parasite. We then initialise the hyperparasite-free system at its eco-evolutionary attractor, and introduce hyperparasites at an arbitrarily low population density. We use a fourth order Runga-Kutta method to solve the ordinary differential equations over a long time period, stopping when the populations have relatively small changes in size, or the time threshold has been reached.

After removing phenotypes that fall below an arbitrary threshold, we choose one of the extant parasite phenotypes (using a weighted probability based on parasite density) and introduce a rare mutant a small phenotypic distance away at a low frequency. We then use this new population as the initial condition for our ordinary differential equation system, which we again evaluate using a fourth order Runga-Kutta method. All code used to produce the figures within this paper is available within the Supplementary Material and on GitHub (GitHub - JasonRWood/Wood_Ashby_2023).

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Measuring the impact of the hyperparasite on the host population

We assume that the hyperparasite is introduced into a well-established host-parasite system, such that the parasite is initially at its continuously stable strategy α_0 (see *Supplementary materials*) and the system is at equilibrium. In addition to exploring the evolutionary implications for parasite virulence we also consider the ecological implications for the host. Specifically, we consider multiple metrics to encapsulate relative disease burden following the introduction of the hyperparasite, namely impacts on: host population size, disease prevalence (proportion of hosts infected), and mortality rates of infected individuals and the overall host population. These measures are formulated below, where S_0, I_0 and N_0 correspond to the steady state of the population before the introduction of the hyperparasite, and asterisks indicate the population with the hyperparasite present (usually evaluated at a continuously stable strategy, α^*).

We measure the relative host population size following the introduction of the hyperparasite as $\Delta_N = \frac{N^*}{N_0}$ and the relative disease-associated mortality rate of infected hosts (Δ_M) is,

$$\Delta_M = \frac{\frac{\alpha^* I^* + \lambda\alpha^* H^*}{I^* + H^*}}{\frac{\alpha_0 I_0}{I_0}} = \frac{\frac{\alpha^*(I^* + \lambda H^*)}{I^* + H^*}}{\alpha_0}.$$

Results

Hyperparasite-induced shifts in virulence evolution

Following the introduction of the hyperparasite, we see pronounced shifts in the evolution of virulence depending on the extent to which hyperparasites modify parasite infectivity (η) and/or virulence (λ), and the extent to which co-transmission of the hyperparasite (ρ) occurs. When the hyperparasite causes hypovirulence (a reduction in the disease-associated mortality rate, $\lambda < 1$), the parasite always experiences selection for higher baseline virulence (Fig. 1a). However, the picture is

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more complicated when the hyperparasite either causes no change to virulence ($\lambda = 1$; Fig. 1b) or induces hypervirulence ($\lambda > 1$; Fig. 1c). Specifically, when the hyperparasite strongly reduces parasite transmission ($\eta \ll 1$), the parasite evolves higher virulence, and when the hyperparasite increases transmission ($\eta > 1$), virulence either remains virtually unchanged ($\lambda = 1$; Fig. 1b) or evolves to lower levels ($\lambda > 1$; Fig. 1c). Yet at intermediate values, there is a hysteresis effect which becomes more prominent as the virulence modifier (λ) or probability of co-transmission (ρ) increase (Fig. 1b-c).

The hysteresis effect (the system demonstrates different behaviour as a parameter is smoothly varied from high to low or from low to high) means that the system can have two evolutionary attractors, and therefore small changes in the underlying ecology (e.g. effects of the hyperparasite on parasite virulence or infectivity) may cause sudden shifts between high and low virulence states that are difficult to reverse (Fig. 2). For example, suppose the hyperparasite causes an increase in virulence ($\lambda > 1$; Fig. 1c), co-transmits with high probability ($\rho \approx 1$), and halves the transmissibility of the parasite ($\eta = 0.5$). Selection would then favour a reduction in evolved virulence relative to the absence of the hyperparasite ($\alpha^* < \alpha_0$) (Fig. 2). A small reduction in the infectivity modifier below a critical threshold, η_c^1, would suddenly shift selection for increased virulence ($\alpha^* > \alpha_0$), but a reversion in the infectivity modifier to its initial value ($\eta > \eta_c^1$) would not lead to a drop in virulence until a second critical threshold, $\eta > \eta_c^2 > \eta_c^1$ is breached (Fig. 2). Thus, relatively small changes in the effects of the hyperparasite on parasite transmission can lead to large changes in the evolution of virulence that may be difficult to reverse.

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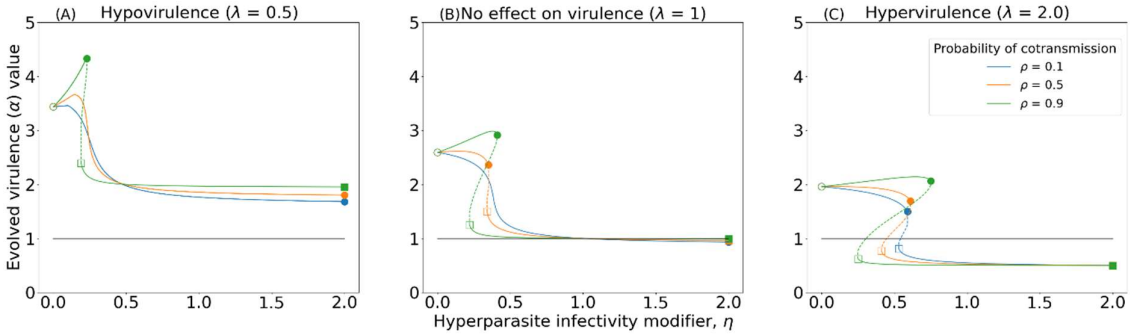

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194 *Figure 1: Evolutionary consequences for parasite virulence (solid: attractors, dashed: repellers) as the effects of the*

195 *hyperparasite on parasite infectivity (hypoinfectivity:* $\eta < 1$*; hyperinfectivity:* $\eta > 1$*) and virulence* (λ) *vary. (A)*

196 *hypovirulence* ($\lambda < 1$)*; (B) no effect on virulence* ($\lambda = 1$)*; (C) hypervirulence* ($\lambda > 1$)*. All panels contain three sets of curves*

197 *showing the evolved outcome as the probability of hyperparasite co-transmission varies:* $\rho = 0.1$ *(blue),* $\rho = 0.5$ *(orange),*

198 $\rho = 0.9$ *(green). The start and end of the hysteresis section are shown with empty and filled shapes. The black line indicates*

199 *the ancestral state prior to the introduction of the hyperparasite. (A-C) Evolved levels of virulence.*

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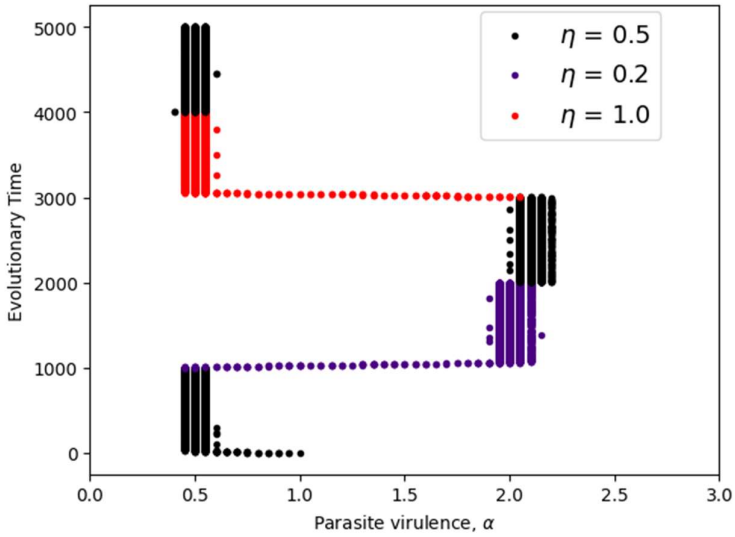

Figure 2: Evolutionary simulations showing the hysteresis affect observed in Figure 1 due to variation in the effects of the hyperparasite on parasite infectivity, η. A relatively small shift (purple) from an initial value of η (black) leads to a sharp increase in virulence. However, a reversion to the initial value of η (black) does not lead to a return to lower virulence. Instead, virulence does not change significantly until a critical threshold is reached (red). Parameters as in Table 1 except $\lambda = 2.0, \rho = 0.9$.

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Effects of hyperparasitism on the host population

Although the parasite may evolve higher or lower baseline virulence following the introduction of the hyperparasite, whether this has a net positive or negative effect for the host population depends on not only the new level of virulence, but also the effects on parasite prevalence, the prevalence of hyperparasites, and the effects of hyperparasitism on virulence. We therefore consider the ecological consequences of the hyperparasite on the host following evolutionary shifts in parasite virulence.

Intuitively, when the hyperparasite causes an evolutionary increase in virulence while not inducing hypovirulence, the burden on infected hosts (i.e. the average disease-associated mortality rate) is always higher (Fig. 3B-C) and the resulting host population size is lower (Fig. 3E-F). This is especially pronounced when the hyperparasite strongly reduces transmission ($\eta \ll 1$). However, the burden on hosts is less straightforward when the hyperparasite induces hypovirulence while selecting for higher virulence (Fig.3A,D), or induces hypervirulence while selecting for lower virulence (Fig. 3C,F). For example, the latter scenario can lead to little effect on the mortality rate of infected hosts (Fig. 3C) but a marked increase in the host population size (Fig. 3F).

Crucially, the hysteresis effect in the evolution of virulence has a striking effect on the host population, with relatively small shifts in the effects of hyperparasites on transmission (η) or on the probability of co-transmission (ρ) causing substantial changes in the relative mortality rate of infected hosts (Fig. 3B-C) and the host population size (Fig. 3E-F). While the hysteresis effect separates a region of lower host population size from a region of higher host population size (Fig. 3D-F), this does not hold for the burden on infected hosts (Fig. 3A-C). Typically, infected hosts either experience a significant increase in average disease burden (for sufficiently low values of η), or there

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231 is little effect. This suggests that the introduction of a hyperparasite rarely improves the outcome of
232 infection on average, even if it is beneficial at the population level by reducing the proportion of the
233 population that is infected. We also see that as the probability of coinfections (ρ) increases, the
234 effect of introducing the hyperparasites on the average disease burden for infected hosts and on the
235 host population size typically grows stronger.

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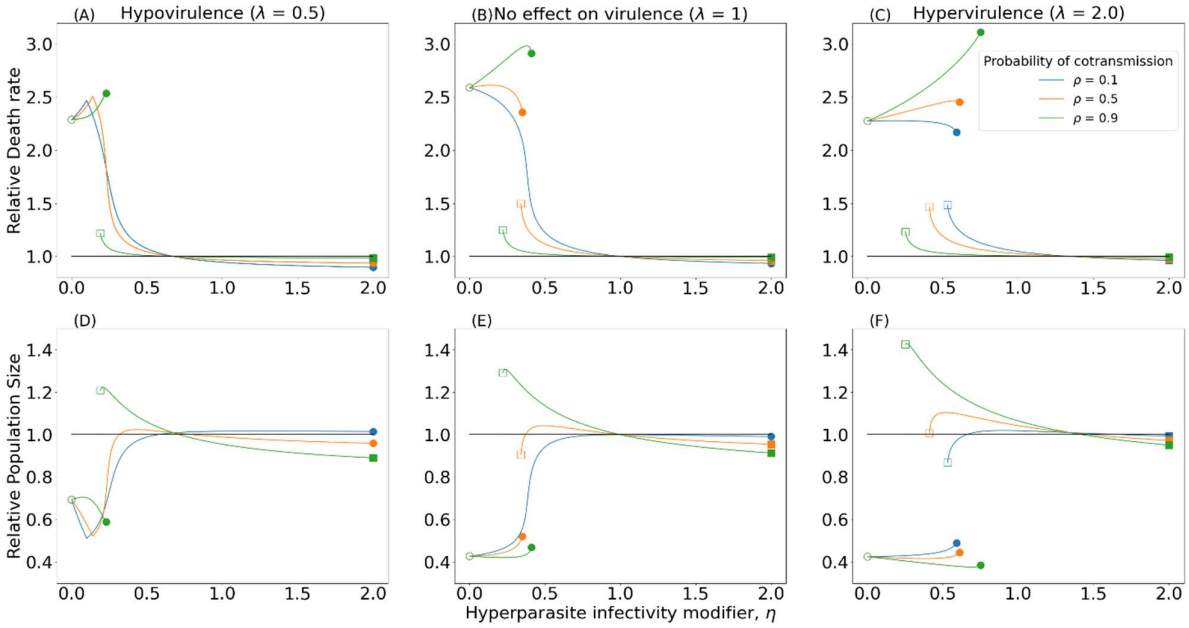

Figure 3: Ecological consequences of hyperparasitism for the host as the effects of the hyperparasite on parasite infectivity (hypoinfectivity: $\eta < 1$; hyperinfectivity: $\eta > 1$) and virulence (λ) vary. Left column: hypovirulence ($\lambda < 1$); middle column: no effect on virulence ($\lambda = 1$); right column: hypervirulence ($\lambda > 1$). All panels contain three sets of curves showing the evolved outcome as the probability of hyperparasite co-transmission varies (shown in Fig. 1): $\rho = 0.1$ (blue), $\rho = 0.5$ (orange), $\rho = 0.9$ (green). The start and end of the hysteresis section are shown with empty and filled shapes. The black line indicates the ancestral state prior to the introduction of the hyperparasite. (A-C) Average mortality rate of infected hosts relative to before the introduction of the hyperparasite. (D-F) Host population size relative to before the introduction of the hyperparasite.

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Hyperparasite-induced diversification in parasite virulence

In the absence of the hyperparasite, parasite virulence evolves to a continuously stable strategy due to the trade-off with transmission, which results in diminishing returns for the parasite. The introduction of the hyperparasite either shifts the continuously stable strategy to a new value (as in Fig. 1) or causes the parasite population to diversify through evolutionary branching into two strains, one with relatively high virulence and the other with relatively low virulence (Fig. 4). In general, branching is most likely to occur when the hyperparasite causes hypervirulence ($\lambda > 1$) and strong hypoinfectivity ($\eta \ll 1$), and the probability of co-transmission is not too high ($\rho \ll 1$) (Fig. 5). When hyperparasites increase virulence and reduce the infectivity of parasites, two contrasting strategies can coexist. The first is a "short-lived", more virulent strain which infects as many hosts as possible before being itself infected by the hyperparasite (green branch, Fig. 4). The second is a "long-lived", less virulent strain which prioritises host infections whilst hyperparasitised (orange branch, Fig. 4).

However, even though one of the parasite strains is less virulent than the other, both are more virulent than the ancestral strain in the absence of the hyperparasite (Fig. 4a). The more virulent strain is rarely infected by the hyperparasite (Fig. 4d), as the host either dies prior to encountering the hyperparasite, or experiences hypervirulence and so dies shortly after being hyperparasitised. Despite the parasite branching into two distinct strategies that are both more virulent than the ancestral state, the overall impact on host and parasite population sizes is minimal (Fig. 4b-d).

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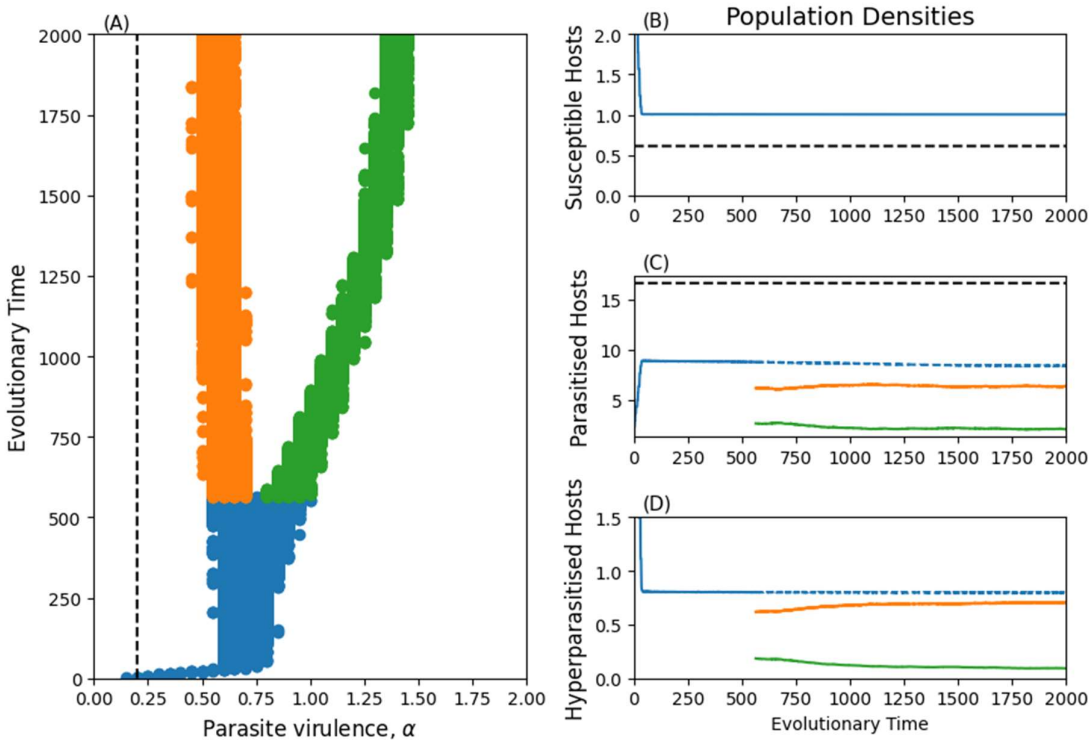

Figure 4. Evolutionary branching in parasite virulence following the introduction of the hyperparasite. Parasite virulence in the absence of the hyperparasite is shown by the dashed line in (A). The parasite branches into relatively high (green) and low (orange) virulence strains. (B-D) Densities of the host, parasite and hyperparasite populations: total (blue), high-virulence strain (green), low-virulence strain (orange). The density of the host and parasite populations in the absence of the hyperparasite are plotted with a dashed black line. *Parameters as in Table 1 except* $d = 0.1, \sigma = 0.4, \rho = 0.5, \eta = 0.25, \lambda = 5.0, \gamma = 0.1$.

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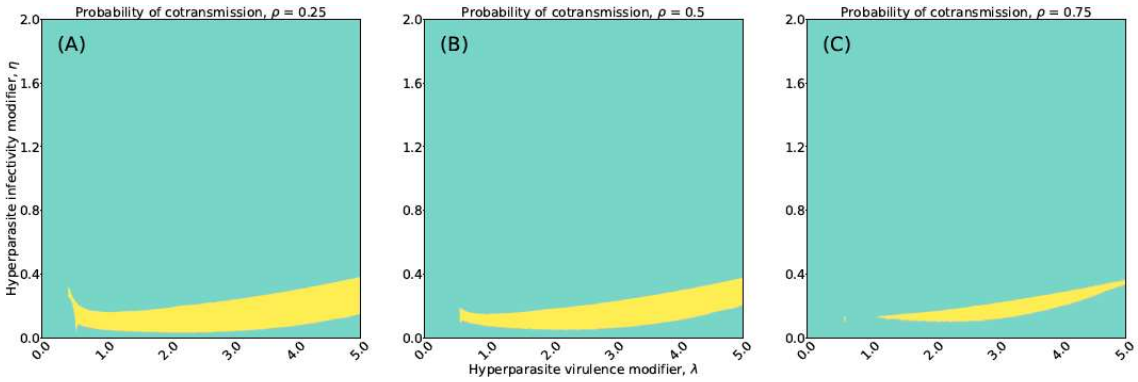

Figure 5. Regions where evolutionary branching occurs (yellow) for the parasite following the introduction of the hyperparasite, as the effects on transmission (η) and virulence (λ) vary. Parameters as in Table 1 except $d = 0.1, \sigma = 0.4, \gamma = 0.1$.

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DISCUSSION

Hyperparasites are abundant in nature and are promising sources of biological control in industry (e.g. food production) and public health (e.g. phage therapy to combat antimicrobial resistance). Yet the eco-evolutionary effects of hyperparasitism are poorly understood. In this study, we have theoretically explored the impact of hyperparasites on the evolution of parasite virulence and the consequences for the host population. We have shown that the introduction of hyperparasites can lead to either higher or lower levels of evolved virulence depending on the ecological effects of the hyperparasite on the parasite. Crucially, we have shown that relatively small changes in the ecological effects of hyperparasites can cause large shifts in virulence, which can be difficult to reverse due to hysteresis. Although a hyperparasite may cause selection for higher parasite virulence, we have shown that this is not necessarily negative for the host population overall, as the hyperparasite can suppress parasite prevalence and may mitigate virulence in infected hosts. Finally, our model shows that the introduction of a hyperparasite can induce diversification in the parasite population, with the potential for both strains to evolve to higher levels of virulence than in the absence of the hyperparasite. Overall, our results suggest that the introduction of hyperparasites can have a strong impact on the evolution of parasite virulence and that the nature of the outcome depends crucially on how the hyperparasite affects parasite virulence and transmission.

Higher parasite virulence typically evolves under two conditions: either the hyperparasite dramatically reduces the infectivity of the parasite population relative to the effect on parasite virulence ($\eta < \lambda$ and $\eta < 1$), or the hyperparasite reduces the realised virulence when hosts are infected ($\lambda < 1$). The former scenario selects for higher virulence because the transmission rate from hyperparasitised hosts is reduced to a greater extent than realised virulence, which shifts the balance in the transmission-virulence trade-off to greater transmission. In the second scenario ($\lambda < 1$), realised virulence is lower in hyperparasitised hosts and so the increased transmission comes at a

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reduced cost. This can be trivially understood by considering a hyperparasite that completely mitigates virulence ($\lambda = 0$), which greatly benefits the parasite. Conversely, when $\lambda > 1$ the hyperparasite increases realised virulence and therefore selects for lower intrinsic virulence.

The hysteresis effect in our model suggests that not only might relatively small changes in hyperparasite traits cause large evolutionary changes in the parasite, but that these might be difficult to reverse. Hysteresis arises because for certain regions of parameter space the system is bistable, meaning that the parasite can evolve to have relatively high or low virulence depending on the current level of virulence. The hysteresis effect can be understood due to changes in the prevalence of the hyperparasite. When the hyperparasite causes a significant reduction in transmission ($\eta \ll 1$) it is very costly to the parasite and so selection favours higher transmission and virulence to avoid being hyperparasitised (upper branch). The hyperparasite is therefore at relatively low prevalence. As the effects of the hyperparasite on infectivity weaken (η increases), the hyperparasite may become more common if it can co-transmit well with the parasite (high ρ), resulting in a gradual increase in hyperparasite prevalence. Eventually a threshold is reached where the risk of hyperparasitism is sufficiently high and the cost sufficiently low that selection favours a large reduction in parasite virulence (lower branch), which feeds back to further increase hyperparasite prevalence. This positive feedback, resulting in high hyperparasite prevalence, is crucial to the hysteresis effect. If the hyperparasite once again reduces parasite infectivity more strongly (η decreases), the system does not return to the high virulence state because hyperparasite prevalence remains high (lower branch). As η continues to fall so too does hyperparasite prevalence, until eventually the risk of hyperparasitism is sufficiently low and the cost sufficiently high that selection favours a large increase in parasite virulence (upper branch).

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The hysteresis effect has especially important implications for biocontrol, as a relatively small change in the hyperparasite can have a large effect on the evolution of virulence and on the host population. While hysteresis is well documented in the ecological literature (e.g. the spruce budworm (Ludwig, Jones, and Holling 1978)), few examples exist in evolutionary models (Prado et al. 2009). As an exception, Prado *et al.* 2009 observed hysteresis when considering the evolution of host sociality and pathogen virulence within contact networks. Prado *et al.* observed that the cycling behaviour they see within their system, has hysteresis-like behaviour where selection for or against host sociality does not occur until the parasite passes critical thresholds.

Our model reveals that the introduction of a hyperparasite can cause disruptive selection leading to diversification into relatively high and low virulence parasite strains (although the "low" virulence strain may still be more virulent than the ancestral strain, as in Fig. 4). Branching typically requires the transmission-virulence trade-off to be close to linear as strongly diminishing returns have a balancing effect on selection. Branching also typically requires the hyperparasite to reduce the infectivity of the parasite far more than it decreases virulence ($\eta \ll \lambda$), which facilitates the existence of high and low virulence phenotypes by creating distinct ecological niches. The high virulence phenotype is rarely infected by the hyperparasite, favouring a "live fast, die young" strategy, while the less virulent phenotype has a longer infectious period to mitigate the burden of the hyperparasite.

Our study is closely related to previous theoretical explorations of evolution in host-parasite-hyperparasite systems (Sandhu et al. 2021; Northrup et al. 2021). Sandhu et al (2021) also explored the evolution of parasite virulence, but in contrast to our study found that the introduction of a hyperparasite generally selects for increased virulence and reduces the average mortality rate of the host. Furthermore, Sandhu et al (2021) did not observe a hysteresis effect, nor did they find

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351 diversification in their model of virulence evolution. The differences in our results are likely due to
352 crucial differences in our assumptions. In particular, we assumed that hosts could recover from
353 infection and that the hyperparasite is co-transmitted with a certain probability (ρ) rather than
354 always occurring, as in Sandhu et al. In reality, one would not expect the hyperparasite to always co-
355 transmit with the parasite, and our model reveals that this parameter has critical effects on the
356 evolution of virulence. Although models of hyperparasitism are relatively rare, especially in an
357 evolutionary context, some of our key findings are mirrored in models of other tripartite systems.
358 For example, multiple studies have found that introducing an additional species to a host-parasite
359 system can lead to evolutionary branching in the host (Best 2018) or the parasite (Kisdi, Geritz, and
360 Boldin 2013; Best 2018; Smith and Ashby 2022) populations. In a related study that also has
361 relevance to biocontrol, Smith and Ashby (2022) explore how the introduction of a tolerance-
362 conferring defensive symbiont affects the evolution of parasite virulence. They show that even if the
363 defensive symbiont is initially beneficial to the host population, in the long-term it is costly because
364 it always selects for higher parasite virulence. Our results similarly emphasise the complex eco-
365 evolutionary outcomes that can arise following the introduction of a hyperparasite, with potentially
366 disastrous consequences for the host population.

367

368 In established host-parasite-hyperparasite systems, it is difficult to separate the ecological and
369 evolutionary consequences of the hyperparasite. Few empirical studies have therefore explored the
370 effects of hyperparasitism on parasite evolution (Parratt and Laine 2016), and those that do often
371 focus on consequences for antimicrobial resistance (Chan et al. 2016; Burmeister et al. 2020), the
372 acquisition of virulence factors from hyperparasites (Miao and Miller 1999), or pleiotropic effects on
373 virulence due to selection for resistance against hyperparasitism (Castledine et al. 2022). For
374 example, (Evans et al. 2010) showed that strains of the bacteria *Erwinia carotovora ssp. atroseptica*
375 *(Eca)* resistant to the hyperparasite ϕAT1 were less likely to produce rot in potato tubers; Casteldine

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et al. showed that evolution of phage resistance in *Pseudomonas aeruginosa* coincided with the loss of virulence in vitro and in vivo; and (Scanlan and Buckling 2012) showed that coevolution between the bacteria *P. fluourescens* and a lytic phage (φ2) selects for a mucoid phenotype, which is a virulence factor in both lung infections of cystic fibrosis patients and in plant infections. Clearly, the available empirical evidence suggests that hyperparasites can indeed have significant evolutionary effects on parasite virulence, although the precise effects may depend on pleiotropy between resistance and virulence. Our model did not consider resistance to hyperparasitism but understanding how pleiotropy with resistance affects virulence evolution is a critical direction for future theoretical work (Sandhu et al. 2021).

We focused our investigation on the evolution of virulence, but the evolutionary dynamics of the hyperparasite are also likely to be important (Sandhu et al. 2021; Northrup et al. 2021). However, in certain cases the hyperparasite might behave as if it is evolutionarily static (e.g. the repeated application of a particular biocontrol to an agricultural crop), in which case the model analysed here will be especially applicable. Still, future theoretical work should consider how our findings are affected by hyperparasite evolution, and whether the hysteresis in our model could lead to fluctuating selection as in Prado et al. (2009). Additionally, the joint dynamics of resistance and virulence evolution deserve further scrutiny, which could be explored using a resource-allocation model where the parasite can either allocate resources to transmission or defence, with the overall resource “budget” depending on the level of virulence.

Overall, we have shown that the introduction of hyperparasites can have dramatic effects on the evolutionary dynamics of parasite virulence, leading to selection for higher or lower virulence, or to branching, with the outcome critically dependent on the effects of the hyperparasite on infectivity and realised virulence, and on the probability of co-transmission. We have also shown how relatively

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small changes in hyperparasite traits can have dramatic consequences for virulence evolution, which has important implications for the use of hyperparasites as agents of biocontrol.

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Conflicts of interest

The authors declare they have no conflicts of interest

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