Title: Complex life cycles and parasite-mediated trophic cascades drive species coexistence and the maintenance of genetic diversity

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Abstract

Ecological and evolutionary processes shape the dynamics of interacting species, yet the drivers of eco-evolutionary feedback remain elusive. Here, we developed an individual-based model of a coevolving predator-prey-parasite system, in which predators can be infected by trophically transmitted parasites. With this setup we combined host-parasite coevolution with host-predator interactions, hence integrating evolutionary and ecological processes. We show that species coexist when parasite-mediated selection is weak on both the predator and the prey. The abundance and genotypic diversity of the hosts are highest at the intermediate parasite-mediated selection on the predator but not on the prey. Interestingly, we show that the evolution of super-resistant genotypes, where host genotypes have resistant alleles in all loci, is driven by interspecific interactions rather than the abundance of the focal species. Overall, host-parasite coevolution shapes the direction of interspecific ecological interactions which, in turn, determine species coexistence and community diversity in a complex system.

Keywords: eco-evolutionary dynamics, host-parasite coevolution, parasite-mediated trophic cascades, species coexistence, genotypic diversity, individual-based model

Introduction

Parasites are arguably one of the strongest selection pressures, as they drive the evolution of key traits in their hosts, including genetic diversity (Schmid-Hempel, 2021). Parasites also play a key role in ecology and community dynamics being important consumers in food webs (Lafferty & Kuris, 2002). Their biomass can exceed that of top predators (Kuris et al., 2008), and most food web links involve parasite species (Lafferty et al., 2008). They are involved in food web stability and interactions via density-mediated trophic cascades (Brunner et al. 2017) and host manipulation that facilitate their transmission (Lion et al., 2006). In turn, some parasites depend on trophic links to complete their complex life cycles (Lafferty et al., 2008). While often compared, parasite pressure is different from predation in that predators eat multiple whole prey. In contrast, a parasite mostly feeds from a single host individual (Lafferty & Kuris, 2002), hence complexifying community dynamics.

Host-parasite coevolution can be driven by fluctuating selection dynamics, where multiple genotypes coexist, or by arms race dynamics, where recurrent novel genotypes reach near fixation in the population (Tellier et al., 2014). Under the arms race predictions, a genotype's advantage is temporary and depends on the abundance and genotype of the antagonistic counterpart (Retel et al., 2019). From a theoretical point of view, host-parasite coevolution is often described using a gene-for-gene (GFG) framework (Thompson & Burdon, 1992). In this framework, some parasite genotypes can infect a wider range of hosts than others, with certain genotypes exhibiting universal virulence (Buckingham & Ashby, 2022). On the other hand, some hosts are better equipped to fend off a greater variety of parasites (Buckingham & Ashby, 2022). The GFG framework enables the exploration of the emergence of host and parasite supertypes, in which fitness is maximised.

Recent theoretical work shows that population dynamics and specialism drive host-parasite coevolution (Buckingham & Ashby, 2022). Fluctuating selection generates host and parasite diversity, and it is typically attributed to highly specific host-parasite interactions (Agrawal & Lively, 2002). However, there is also evidence suggesting that specificity from the matching-alleles framework is not necessarily required to generate fluctuating selection dynamics and that ecological dynamics is an important contributor (Best et al. 2017). Population dynamics can have significant effects on coevolutionary dynamics (Buckingham & Ashby, 2022) because population sizes ultimately determine the supply of *de novo* mutations (Retel et al., 2019). Particularly, in small population

sizes, demographic fluctuations and genetic drift may lead to extinction (Huang et al., 2015) ending the coevolutionary process.

In natural systems, host-parasite coevolution does not occur in isolation, and both predator and prey can become hosts (Lafferty, 1992; Strona, 2015; Brunner et al., 2017). Experimental evidence shows that the rapid evolution of predators' attack traits and prey's defence traits can influence population dynamics (Yoshida et al., 2003; Hairston Jr. et al., 2005; Becks et al., 2012). Theory further predicts that when predators' attack traits evolve faster than prey's defence traits, predator-prey dynamics result in large-amplitude oscillations of population size (Mougi & Iwasa, 2010). Contrarily, when prey's defence traits evolve faster than predators' attack traits, a stable equilibrium or small amplitude oscillations of population size are likely to occur. While parasites typically evolve faster than the host due to their short generation times as opposed to predator-prey systems (Mougi & Iwasa, 2010), host-parasite dynamics would tend to have large-amplitude oscillations when a single host can be attacked by multiple parasite species (Dawkins & Krebs, 1979; Mougi & Iwasa, 2010). In an experimental setup where predator-prey and host-parasite interactions are integrated, the evolution of prey resistance can maintain species coexistence by allowing the prey population to support both consumers (Frickel et al., 2017). Yet, the influence of multiple species evolution on species coexistence and the maintenance of genetic diversity remains to be fully explored in a general framework.

Individual-based modelling is a powerful tool to simulate such a complex system with integrated coevolving species and allow eco-evolutionary feedback. Specifically, the probabilities of host and parasite or predator and prey encounters depend on populations' densities (Segarra, 2005), which are regulated by species interactions (Ashby et al. 2019; Gutiérrez Al-Khudhairy & Rossberg, 2022). Thus, when species interactions and coevolution impact the ecological dynamics, such as population sizes, this will in turn impact the *de novo* mutation supply. The scholastic effects in individual-based models (IBM) can naturally capture the genetic makeup of populations through mutations (e.g. Huang et al., 2012) and individuals' responses to ecological and evolutionary mechanisms (Romero-Mujalli et al., 2019).

Here, we explored the eco-evolutionary dynamics of a coevolving predator-prey-parasite community using an IBM where the parasite is transmitted trophically (Híjar-Islas et al., 2023; Figure 1). While demographic fluctuations exist due to species interactions, extinction of species naturally happens depending on the variation in resistance and infectivity shaping host-parasite interactions. We investigated the

conditions that lead to species coexistence and examined the effects of parasite-mediated reproductive costs on predators and prey on species abundance (i.e., ecological process) and intraspecific diversity (i.e., evolutionary process). Finally, we analysed the conditions that lead to the evolution of super-resistance and super-infectivity, and whether the selection of these traits is more determined by population size or by the strength of host-parasite interactions.

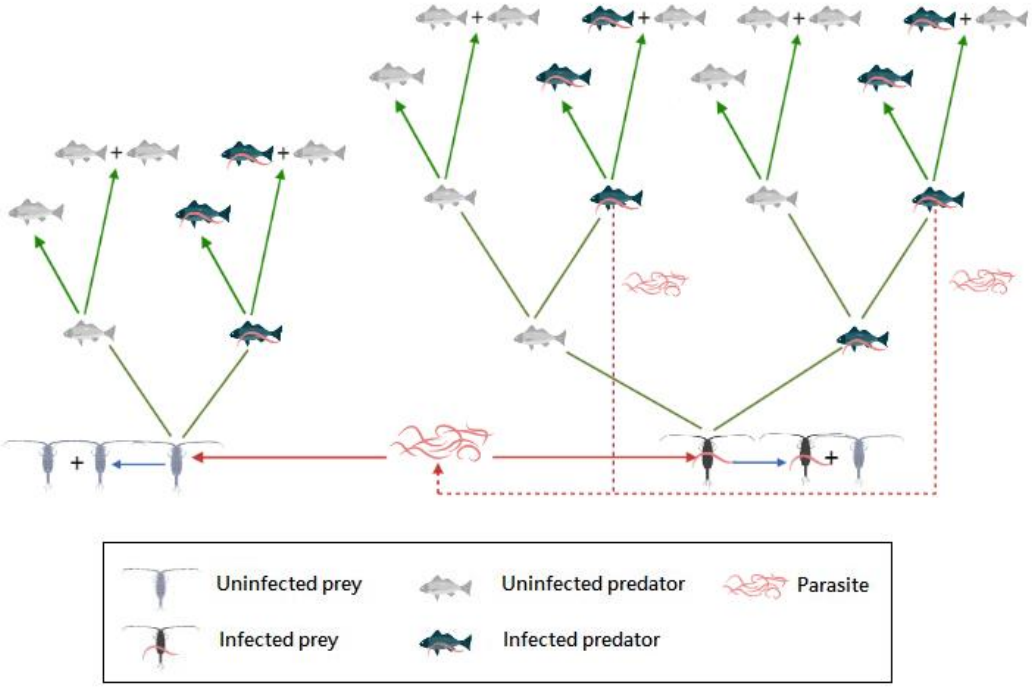

Figure 1. Predator-prey-parasite system. The arrows illustrate all the possible events in the system (red for the parasite, blue for the prey and green for the predator). Note that the parasite can transition from a free-living stage to a state inside the prey (if successfully infecting the prey) until reaching the predator (if successfully infecting the predator), where it reproduces and is released again to a free-living stage to begin a new life cycle. The predator is allowed to get infected multiple times.

Results

We used an IBM of a coevolving predator-prey-parasite system in which the parasite is transmitted trophically from prey to predator and reproduces when successfully infecting the predator (Híjar-Islas et al., 2023). We used a gene-for-gene model for the species interactions (details in Methods), where mutations happen during the reproduction of predator, prey, and parasite species at the rates of μ_y, μ_x, μ_z, respectively. Here, mutation rates are defined per loci per reproduction event. To ensure the three species evolved on similar time scales, we adjusted the mutation

rates according to the population sizes (see details in Supplementary Note 1). We introduced two parameters, r_x and r_p to capture parasite-mediated selection on the prey and the predator, respectively. The values of r_x and r_p range between 0 and 1, where 0 indicates the parasite completely suppresses the host's ability to reproduce, and 1 means no impact on the host's reproduction. We ran multiple independent simulations and investigated how parasite-mediated selection impacts our coevolving system.

Evaluating species coexistence

Consistent with previous findings on a non-evolving predator-prey-predator system (Híjar-Islas et al., 2023), we found that in a coevolving system the coexistence of all species happens under the joint impact of reproductive costs on both prey and predator species (red bars in Figure 2). When the reproductive cost on infected predators is low, there is a high likelihood of all species extinction in a wide range of reproductive costs on infected prey. When the reproductive cost of infected predators is high, we see a significant drop in all species' coexistence with increasing reproductive costs on infected prey. Species coexistence is maximised at low reproductive costs on both infected predators and prey. Due to its complex life cycle, the parasite needs to be transmitted from prey to predators to reproduce.

We observe that the coexistence of only the predator and the prey with the extinction of the parasite is more common with intermediate parasite-mediated selection on the predator (white bars in Figure 2). This suggests the emergence of density-dependent transmission of the parasites, which becomes less likely as the predator population size decreases in response to the cost of infection. Once the parasite goes extinct, the selection of becoming resistant is removed, and the predator population grows again and fluctuates with that of the prey.

With high reproductive costs on infected predators, we show that for a wide range of reproductive costs on prey, the predator is likely to go extinct first, followed by the parasite, and the prey population reaches the environment's carrying capacity (blue bars in Figure 2). On the contrary, with low reproductive costs on infected predators, we only observe the extinction of both predator and parasite with the prey remaining in a very narrow range, i.e., when reproductive costs on infected prey are relatively high. This seems counter-intuitive at first sight. However, if we combine the observation of all species extinction, high reproductive costs on infected prey also drive all species

extinction (purple bars in Figure 2). Thus, two scenarios are happening when reproductive costs on infected predators are low but on infected prey are high. Either the low prey abundance leads to its extinction and then the extinction of all species, or the extinction of the predator first is followed by the parasite, and then the recovery of the prey population.

In addition, the likelihood of all species going extinct increases when the reproductive costs on infected prey remain high but that of infected predators decreases (purple bars in Figure 2). Note, that all-species extinction always happens when the prey goes extinct first. These two observations show that prey extinction is a combined result of high reproductive costs on prey caused by parasites as well as predation pressure, especially when the predator is less impacted by the parasites.

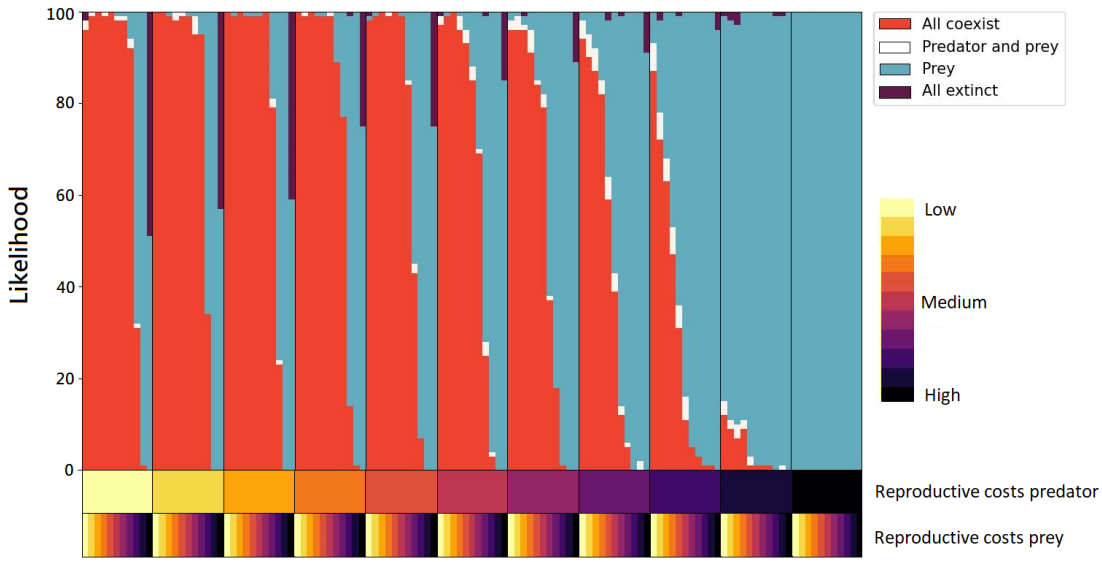

Figure 2. Patterns of coexistence of parasite, prey, and predator. Proportions are taken as a function of parasite-mediated selection on the predator and the prey, from 100 independent realisations. The y-axis shows the number of independent realisations. The x-axis shows the different combinations of parameters r_x (reproductive costs prey) and r_p (reproductive costs predator). Note that the values of r_x and r_p are opposite to the level of reproductive costs. This means a high reproductive cost (marked as darker colors in the x-axis) corresponds to a small value of r_x and r_p. The values of r_x and r_p decrease from left to right in the x-axis from 1 to 0, with an interval of 0.1, i.e., r_x = 1, 0.9, 0.8,…, 0, and r_p = 1, 0.9, 0.8, …, 0.

To further understand why and which of the predator, prey and parasite can coexist, we recorded the species abundances as well as genotypic diversity over time (Figure 3). We examined the relationship between genetic diversity and species abundance. A lack of correlation could suggest the dominance of a supertype in the system, monopolizing the ecological niche and preventing the emergence and spread of other

genotypes (Retel et al., 2019). While most new mutations will fail to invade the coevolutionary system and get lost due to drift, we focused on the effective genotypes making up at least 2% of the population (Figure 3), to avoid the noise due to continuously arising mutations with low frequencies.

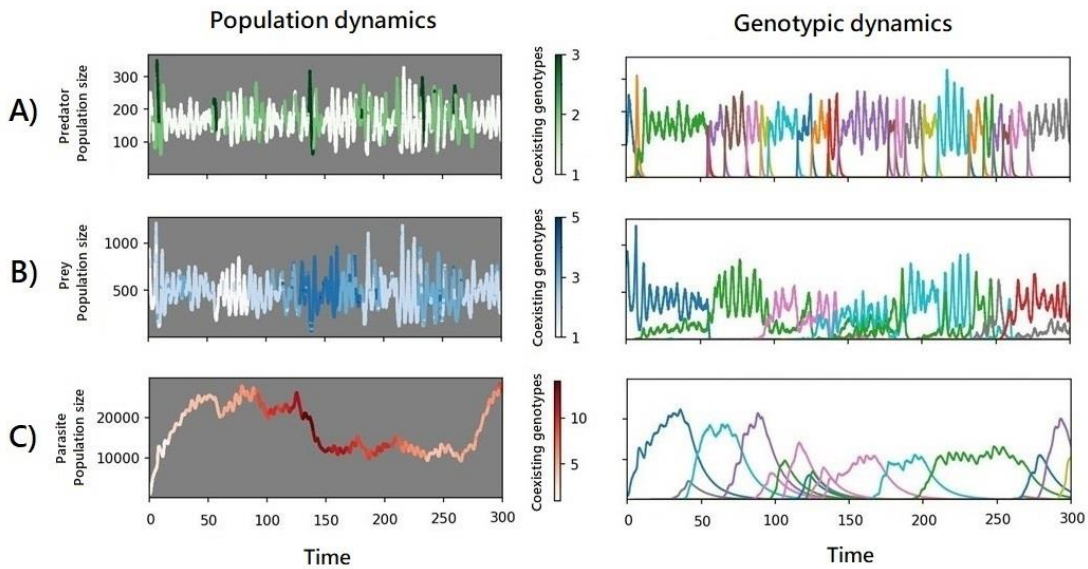

Figure 3. Population and genotypic dynamics from a single realisation. The left panels show the population dynamics of the species, where darker shading indicates a higher number of genotypes coexisting. The right panels show the genotypic dynamics of the species, where different colors show the emergence of different genotypes. A) population and genotypic dynamics of the predator, B) population and genotypic dynamics of the prey, and C) population and genotypic dynamics of the parasite. The parameter space is no reproductive costs on the prey and the predator (i.e., $\boldsymbol{r_x}$ = 1; $\boldsymbol{r_p}$ = 1).

Drivers of population size fluctuation

We found the direction of the correlations between species abundance to be heavily influenced by the strength of parasite-mediated selection on the hosts' fitness (Table S2). Specifically, in the same parameter space, the parameter sets leading to the highest abundance differ for each species, resulting in non-monotonic consumer-resource correlations (Figure 4A-C, Table S2). Predators' abundance is highest in the absence of parasite-mediated selection on both the predator and the prey, which maximises reproduction and high prey availability (upper left corner of Figure 4A).

Prey population size is maximised when parasite-mediated selection on the predator is strong, hence suppressing predator-mediated selection, and there are no reproductive costs on infected prey (lower left corner of Figure 4B). The magnitude of change in the prey population that results from different strengths of parasite-mediated

selection on the prey is small in a parameter space where predators are abundant (Figure S6). This is due to the net effect of both consumers on the prey population: when the prey is not affected by the parasite, there is more prey available for the predator to consume, leading to lower prey abundance. Conversely, with increasing parasite-mediated selection on the prey, there are fewer prey individuals available for the predator to consume, resulting in weaker predation pressures in a bottom-up control. Interestingly, the mean prey population size reaches its lowest level under no parasite-mediated selection on both hosts, suggesting the strength of parasite-mediated selection controls the strength of predation pressure when both exist.

Lastly, we found that parasite population size is maximised with intermediate strength of parasite-mediated selection on the predator combined with no reproductive costs on infected prey (middle left corner of Figure 4C). The reduced parasite-mediated selection on the prey maximises uptake by the intermediate host, while the intermediate cost on the final host maximises predator density.

Overall, different conditions favor the abundance of different species in such a complex coevolving system. Reduced parasite-mediated selection on the prey combined with intermediate levels of selection on the predator, however, not only maintains species coexistence but also maximises species abundances for all species.

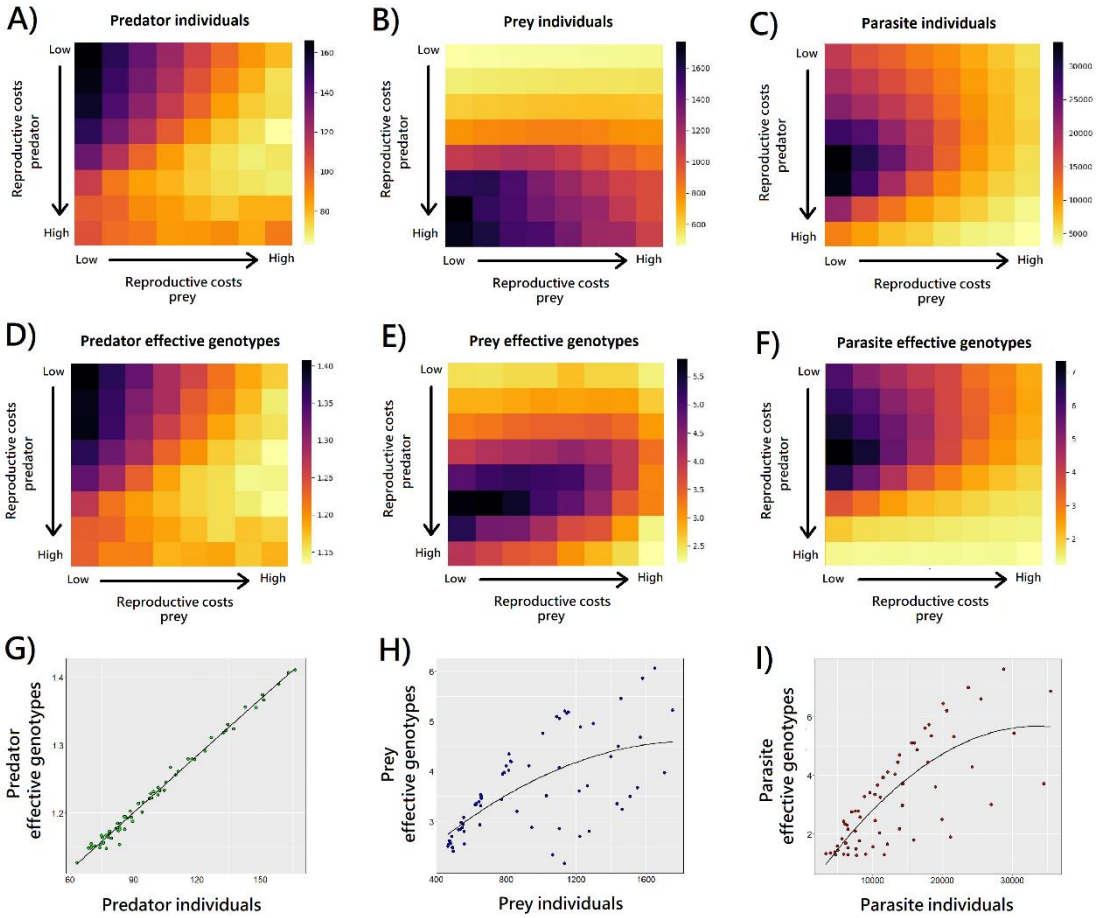

Figure 4. Mean population size and mean genotypic diversity. Values are taken as a function of the strength of parasite-mediated selection on the predator and the prey when the three species coexist. The mean population size of A) predators; B) prey; and C) parasites; The mean genotypic diversity of D) predators; E) prey; and F) parasites. Each panel displays the average value from 100 independent realisations over a time period of 200-1000 time steps for each realisation. The correlation between mean population size and mean genotypic diversity of G) predators; H) prey; and I) parasites. Each dot displays the average value from 100 independent realisations over a time period of 200-1000 time steps for each realisation.

Drivers of genetic diversity

To understand the link between ecology and evolution, we correlated the different population sizes and levels of genetic diversity across replicated realisations. We confirm that genetic diversity is not merely driven by population abundances, where the highest abundance always leads to the highest diversity, especially for the prey and parasites (comparing Figures 4B vs 4E, Figures 4F vs 4C).

We observed that the genetic diversity of predators does increase with population size (linear model: $F_{1,\ 62} = 6{,}469$; $p < 0.0001$; Figures 4D, 4G). Analysis of predator genotypic dynamics revealed consecutive selective sweeps, consistent with the

prediction of an arms race, in which genotypes are unlikely to coexist at high frequencies (Figure 3, Figures S7, S8). With no parasite-mediated selection, the frequency of those selective sweeps is the sole consequence of the mutation rate in large population sizes. When the presence of a parasite imposes strong reproductive costs on the predator population, the size of the predator population decreases, leading to longer and less frequent selective sweeps. In turn, this reduction in predator population size leads to a decrease in the population size of the parasite, resulting in lower infectivity due to a decrease in the supply of mutations.

Surprisingly, we observed that prey genetic diversity is highest under conditions of intermediate reproductive costs on predators and in the absence of parasite-mediated selection on the prey (quadratic regression; $F_{2, 61} = 20.89$; $p < 0.0001$; Figures 4E, 4H). These conditions maximise parasite abundance (Figure 4C), rather than prey abundance (Figure 4B), suggesting that prey diversity is not a result of the prey population size, but instead the result of prey-parasite coevolution. Also, in this parameter space, the parasite controls the predator's reproduction rate, allowing the prey to reproduce more rapidly and potentially leading to higher prey genotypic diversity. The absence of strong selection pressures on the prey allows different prey genotypes to coexist, as resistance does not confer any infection-related advantage among genotypes.

Finally, we found that the parasite genetic diversity increases with parasite abundance in a non-linear manner (quadratic regression; $F_{2, 61} = 45.74$; $p < 0.0001$; Figures 4F, 4I). We also found that in the predator population, the genotypic lifespan is negatively correlated with the abundance (quadratic regression; $F_{2, 61} = 616.9$; $p < 0.0001$; Figure 5A) and with the genotypic diversity (quadratic regression; $F_{2, 61} = 651.6$; $p < 0.0001$; Figure 5D). Similarly, in the parasite population, we found that the genotypic lifespan is negatively correlated with the abundance (quadratic regression; $F_{2, 61} = 19.93$; $p < 0.0001$; Figure 5C) and with the genotypic diversity (quadratic regression; $F_{2, 61} = 179.8$; $p < 0.0001$; Figure 5F). Contrarily, prey genotypic lifespans are longer with medium abundance (quadratic regression; $F_{2, 61} = 3.931$; $p = 0.0248$; Figure 5B) and with medium genotypic diversity (quadratic regression; $F_{2, 61} = 3.049$; $p = 0.0547$; Figure 5E). These trends suggest that strong reciprocal selection pressures between the predator and the parasite lead to the non-coexistence of different genotypes, where advantageous genotypes monopolize the population. While genotypic dynamics are also shaped by selective sweeps, the lifespans of the parasite genotypes are longer than those of the prey and predators, particularly under high reproductive costs on the predator that result in low parasite abundance and low genotypic diversity (Figures S7,

S8). As the parasite needs to reach the predator to reproduce, when it imposes high reproductive costs on the predator, the parasite population size decreases, leading to fewer mutations. If the predator evolves resistance, however, the parasite population persists with low infectivity and coexists with the predator (Figure S8).

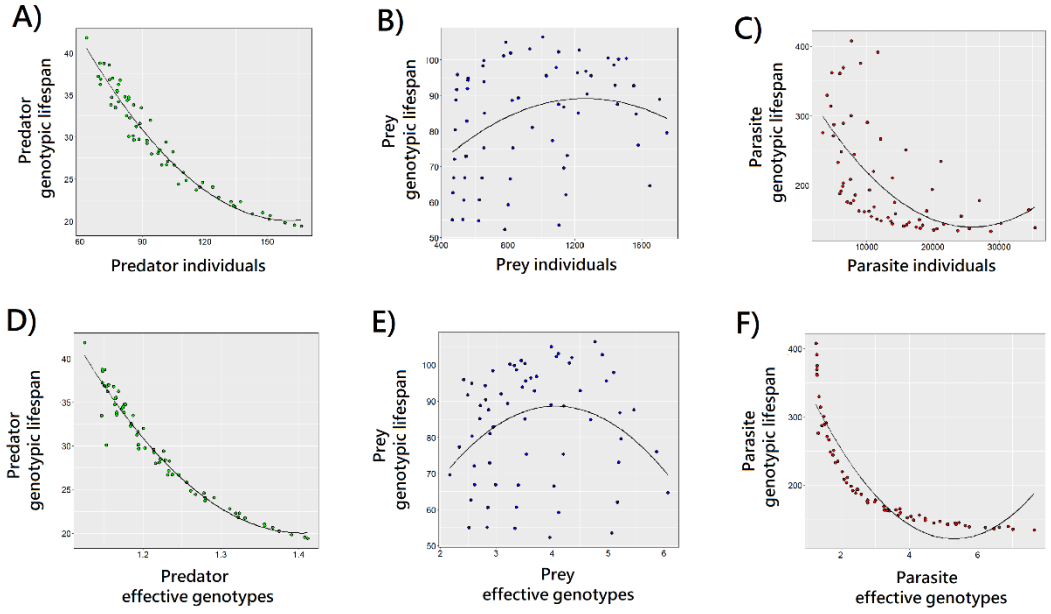

Figure 5. Species' genotypic lifespan according to the population size and genotypic diversity. The top panels show the correlations between mean population size and mean genotypic lifespan of the A) predator, B) prey, and C) parasite. The bottom panels show the correlations between mean genotypic diversity and mean genotypic lifespan of the D) predator, E) prey, and F) parasite. Each dot displays the average value from 100 independent realisations over a time period of 200-1000 time steps for each realisation.

Evolution of super-resistant and super-infective genotypes

Our gene-for-gene coevolving model allows us to detect the emergence of super-resistant prey and/or predator genotypes, as well as super-infective parasite genotypes. A super-resistant host is a host genotype that has resistant alleles in all modelled loci, while a super-infective parasite is a genotype that has infective alleles at all loci. Our results suggest the evolution of these supertypes in a focal species is mediated by the abundance of its interacting species, rather than by its abundance (Figure 6).

When all species coexist, predators are more likely to evolve super-resistant genotypes under conditions of intermediate parasite-mediated selection on the predator combined with no reproductive costs on the prey (11% of the realisations, Figure 6A), which is the parameter space with highest parasite abundance (Figure 4C)

and strongest selection pressures on the predator. The evolution of super-resistant prey genotypes is mostly driven by predator abundance rather than parasite-mediated selection on the prey. This suggests that parasites drive the evolution of prey resistance through the predator, leaving a signature of indirect parasite-mediated selection. Prey are more likely to evolve super-resistant genotypes with intermediate reproductive costs on the predator and the prey due to lower predation pressure (22% of the realisations; Figure 6B). Parasites on the other hand are more likely to evolve super-infectivity under no parasite-mediated selection, particularly combined with intermediate reproductive costs on the predator (30% of the realisations; Figure 6C). This suggests that the probability of the evolution of super-infectivity scales with parasite abundance, and super-infective parasite genotypes are selected only when reproductive costs on both hosts are not high.

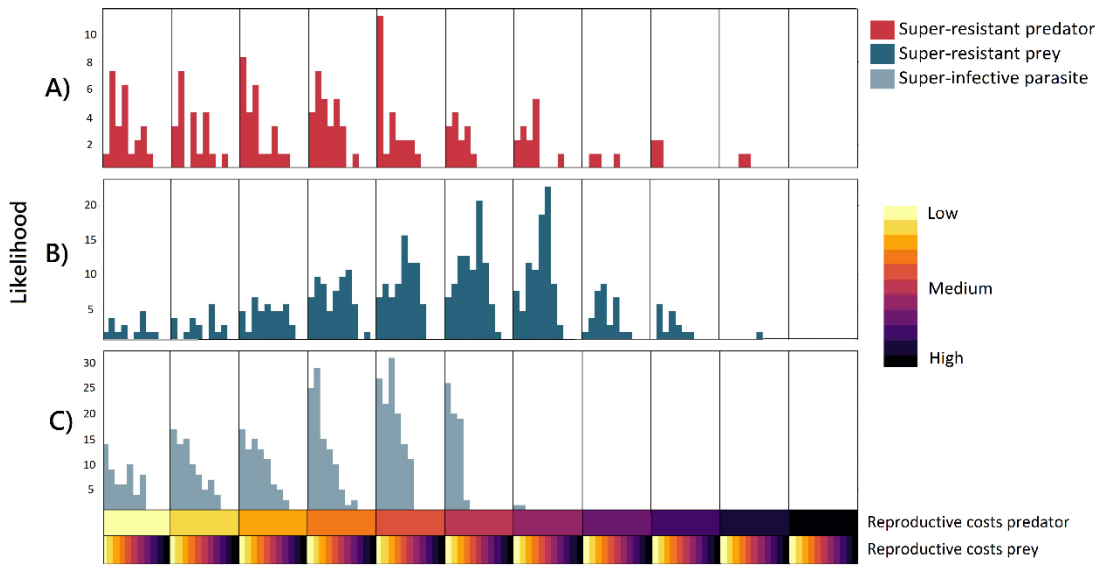

Figure 6. The emergence of supertypes when the three species coexist. A) Evolution of a super-resistant predator genotype; B) Evolution of a super-resistant prey genotype, and C) Evolution of a super-infective parasite genotype. Proportions are taken as a function of the strength of parasite-mediated selection on the predator and the prey, from 100 independent realisations (shown on the y-axis). The x-axis shows the different combinations of parameters r_x (reproductive costs prey) and r_p (reproductive costs predator). Note that the values of r_x and r_p are opposite to the level of reproductive costs. This means a high reproductive cost (marked as darker colors in the x-axis) corresponds to a small value of r_x and r_p. The values of r_x and r_p decrease from left to right in the x-axis from 1 to 0, with an interval of 0.1.

Discussion

While it is well known that the dynamic interplay of ecological and evolutionary processes shapes interspecific interactions, the role of these eco-evolutionary

dynamics in complex systems such as food webs remains to be generalised. By integrating host-parasite and predator-prey interactions in a coevolving system with mutations, we demonstrate that species coexistence is more likely under low parasite-mediated selection on both host species, while abundance and genotypic diversity increase with intermediate parasite-mediated selection on the predator combined with no reproductive costs on the prey. Our results show that consumer-resource correlations are non-monotonic and determined by the strength of parasite-mediated selection and the hosts' fitness. We also show that multiple interspecific interactions drive the emergence of supertypes, in which host and parasite fitness is maximised. Overall, our findings suggest that species coexistence and community diversity are mediated by host-parasite coevolution, which is shaped by multispecies ecological interactions.

We show that parasite-mediated selection determines species' coexistence. In our coevolving predator-prey-parasite system, the prey is a shared resource for parasites and predators. Due to this niche overlap, the prey goes extinct under strong parasite and predator-mediated selections, ultimately leading to the extinction of all three species. The predator is a consumer of the prey and a resource of the parasite, so a large predator population size is beneficial for the parasite but detrimental to the prey. With few prey individuals, the predator population collapses, followed by the parasite population. The parasite remains if there is enough prey for the predator to consume and enough predators to act as final hosts. Under strong parasite-mediated selection, the evolution of resistance rescues the system and allows species coexistence. This theoretical prediction matches the experimental demonstration of the rescue mechanism detected in virus-host-predator systems, where host-virus coevolution facilitates the coexistence of competing consumers (Frickel et al., 2017).

In bacterial-phage systems, phages evolve faster than their bacterial host (Campbell, 1961; Heilmann et al., 2010; Rudolf & Antonovics, 2005). Due to their rapid evolution, phages can coexist with their bacterial host through ecological mechanisms such as reduced phage dispersal (Brockhurst et al., 2006), and density-dependent spatial bacteria refuges (Heilmann et al., 2012). In our system, parasite infectivity evolves at the same pace as host resistance because of the parasite's complex lifecycle. The evolution of resistance in the predator can be advantageous for the parasite under strong parasite-mediated selection on the predator, and the three species can coexist with the predator and parasite remaining at small population sizes.

When integrating parasites in food webs, predator-prey correlations are non-monotonic and depend on the strength of parasite-mediated selection, which maximises predator and prey abundance as well as genotypic diversity. Parasites influence predator-prey interactions via predator-mediated effects (top-down cascade) on the prey and prey-mediated effects (bottom-up effect) on the predator (Anaya-Rojas et al., 2019). The abundance of the three species is highest under no parasite-mediated selection on the prey and with intermediate reproductive costs on the predator given that the predator is a resource to the parasite and a consumer to the prey. This parameter space maximises the abundance of the three species. This prediction matches observation where parasites that use multiple hosts to complete their life cycles often impose relatively no harm to the intermediate host and are very harmful to the final host in which they reproduce (De Castro & Bolker, 2005; Scholz et al., 2021). Interestingly, we find that the prey is more abundant under strong parasite-mediated selection on both hosts than under no parasite-mediated selection. This suggests that parasites control prey abundance via indirect effects on the predator population and that the maintenance of genetic diversity is driven by multiple selective agents (Gotanda et al., 2013).

The number of genotypes at a given time point reflects the genetic diversity of the focus species. In conditions where selection pressures are strong, a genotype may dominate the ecological niche due to evolutionary-mediated priority effects (De Meester et al., 2016). Contrarily, when selection pressures are weak, more genotypes are likely to co-occur and drift neutrally (De Meester et al., 2016). In our system, genotypic diversity increases in the absence of parasite-mediated selection on the hosts because the evolution of resistance alleles confers no fitness advantage. On the other hand, the lifespan of a genotype reflects its dynamics and the sweeps it undergoes when intraspecific diversity is low. The lifespans of the genotypes are determined by the population size because the mutation supply increases with population size and provides more fuel for adaptation (Retel et al., 2019). Even under weak parasite-mediated selection, host-parasite interactions can lead to rapid allele frequency shifts, even those initially rare (Gokhale et al., 2013), as demonstrated experimentally in three-spined stickleback for instance (Eizaguirre et al., 2012).

We show that host and parasite genotypic dynamics are shaped by consecutive selective sweeps, where the lifespans of the parasite genotypes are longer than those of the prey and predators. These genotypic dynamics have been shown experimentally in a host-virus system (Retel et al., 2019). Interestingly, we see that the predator genotypic dynamics are governed by the succession of many selective sweeps rather

than coexisting genotypes, even in the absence of parasite-mediated selection. This suggests that predator-prey interactions alone can facilitate the rapid emergence of resistance genotypes. Note that since we use a haploid genetic system, mutations are directly exposed to selection. The fitness advantage of those mutations depends on the allelic states of co-occurring host-parasite genotypes. Our results suggest that recurrent selective sweeps are common in the predator population and shape long-lasting coevolutionary dynamics with the parasite.

In host-parasite interactions, population and allele dynamics interact to determine coevolution (Retel et al., 2019). Our results show that host-parasite coevolution is influenced by other species interactions. For instance, both predator and parasite-mediated selections drive the evolution of super-resistant prey genotypes. Indeed, we show the prey is more likely to evolve super-resistant genotypes under relaxed predation pressure that leads to high population size, and hence large mutation supply. Under those conditions, the presence of parasites selects for resistant genotypes in the prey population. We also see that in the simulations where the predator and parasite go extinct, the prey population does not evolve super-resistant genotypes as resistance does not provide any advantage in a parasite-free system. Super-infective parasite genotypes evolve under low parasite-mediated selection on both hosts because high infectivity leads to host extinction when the parasite decreases largely its host fitness. The parasite is more likely to evolve super-infectivity under a large population size, which is exactly the parameter space where the availability of both hosts increases. As the predator is more likely to evolve super-resistant genotypes when parasites are abundant, the parasite needs to counter-evolve infectivity to persist and complete its lifecycle.

We know that host-parasite coevolution is determined by the population dynamics and genetic makeup of interacting species (Buckingham & Ashby, 2022). Here, we demonstrate that, in a complex system, host-parasite coevolution is also shaped by interspecific interactions, where prey-parasite interactions are altered via top-down control by the predator, and predator-parasite interactions are mediated by the prey in a bottom-up manner. Host-parasite coevolution, in turn, shapes population dynamics, as the evolution of resistance mitigates the detrimental effects on the hosts. The predator and/or prey population size changes, that result from parasite-mediated selection, hence, are associated with evolution-mediated trophic cascades that impact the whole community's abundance and diversity. This has been demonstrated experimentally, in a non-microbial system, where the evolution of resistance in predator fish impacts prey community structure (Brunner et al., 2017). This dynamic interplay of

ecology and evolution becomes evident at different strengths of parasite-mediated selection on the hosts. The degree of these effects determines the magnitude of change in population sizes which, in turn, determines the evolution rates for resistance/infectivity shaping host-parasite interactions.

Methods

Model of host-parasite coevolution

We constructed the host-parasite coevolution by extending a multi-locus gene-for-gene model (Sasaki, 2000). We consider a prey (i.e., intermediate host) genotype for resistance $j = j^1, j^2 \ldots j^n$, a predator (i.e., final host) genotype for resistance $l = l^1, l^2 \ldots l^n$, and a trophically transmitted parasite genotype for infectivity $k = k^1, k^2 \ldots k^n$, where n corresponds to the number of loci (i.e., genotype length). X^j is the prey of genotype j, Y^l is the predator of genotype l, and Z^k is the parasite of genotype k, where j, l and k are integers from 0, 1, 2… to infinity if the host-parasite system evolves forever. For different genotypes, the allelic state of prey resistance, predator resistance, or parasite infectivity, at each locus of the genotypes is a binary number, where '0' corresponds to susceptible/non-infective alleles and '1' to resistant/infective alleles. These allelic states are reversible, where mutations can lead to allelic changes in both directions for all the genotypes (i.e., from '0' to '1', or from '1' to '0').

For simplicity, we focus on haploid and asexual hosts and parasites. The parasite genotypes possess 10 loci for infective/non-infective alleles, and both the prey and predator hosts genotypes possess 10 loci for resistant/susceptible alleles. Random mutations arise in the three species after a reproduction event with rates μ_x for the prey, μ_y for the predator, and μ_z for the parasite. μ_x, μ_y, μ_z, are values between 0 and 1, i.e., the mutation rates per locus per reproduction. To simulate the mutation event of any genotype per reproduction, we draw a random number between 0 and 1 for each locus. If any n random number is smaller than the mutation rate, a mutation happens, otherwise, the genotype is the same as the parent.

All the hosts (predator and prey) are initially susceptible, and all parasites are initially non-infective except to the initial hosts (i.e., all genotypes are initially simulated as strings of '0'). If the genotype of the initial prey X^j is 00…00, and the genotype of the initial parasite Z^k is 00…00, then the initial effective resistance is $r(X^j, Z^k) = 0$, and the probability of successful infection of the initial parasite to the initial prey is

$Q_x(X^j, Z^k) = 1$. Likewise, if the genotype of the initial predator Y^l is 00…00, and the genotype of the initial parasite Z^k is 00…00, then the initial effective resistance is $r(Y^l, Z^k) = 0$ and the probability of successful infection of the initial parasite to the initial predator is $Q_y(Y^l, Z^k) = 1$ (see details of the probability of successful infection in Supplementary Note 2).

We consider well-mixed populations, where the encounter rates between the hosts and parasites are density dependent. Ultimately, the probability of successful infection between a parasite and a host is a function of the allelic states across all loci of the parasite and the host genotypes. We consider each position in the host genotype to see whether it is resistant to a particular locus of the interacting parasite. The concrete value of host resistance r is the number of resistant alleles in the host genotype ('1') matching the same position as non-infective alleles in the respective parasite genotype ('0').

The individual-based model

We used an IBM of a predator-prey-parasite system, where the parasite is transmitted trophically from infected prey to predators (Híjar-Islas et al., 2023). We allowed a coevolving predator-prey-parasite community, where mutations can happen in the prey, predator, and parasite genotypes in reproduction. The allelic states across all loci of the predator, prey and parasite genotypes ultimately determine the probability of successful infection. Each individual-level event occurs with a type-specific rate indicated by the parameters above the arrows in our reactions. The model parameters are shown in Table S1 and the choice of parameter values is shown in Supplementary Note 1. While reactions and their rates are defined at the individual level, how often e.g., reproduction of prey happens at the population level will also depend on the population size.

Possible events only in the prey population

X_k^j is the prey population size of the genotype j. When $k = 0$, the prey does not carry a parasite. Suppose such a prey reproduces an offspring X_0^a. The new offspring has the same genotype as the parent when $a = j$, and is a mutant when $a \neq j$. The probability of no mutation happening during a reproduction event is $(1 - \mu_x)^n$, where $\mu_x \in [0,1]$ is the probability of a mutation per locus and n is the number of loci in the

genotype. We note here that the prey offspring is parasite-free and can be infected by the free living-parasite population in the environment.

In the absence of the other species the prey populations can be described by the following reactions, where $g_x \in R^+$ is the reproduction rate of the prey, $r_x \in [0,1]$ is the suppression of an infected prey reproduction, $K \in R^+$ is the environmental carrying capacity of the prey population, and $d_x \in R^+$ is the intrinsic death rate.

$$X_0^j \xrightarrow{g_x} X_0^j + X_0^a$$

$$X_k^j \xrightarrow{r_x * g_x} X_k^j + X_0^a$$

$$X_k^j + X_k^j \xrightarrow{\frac{1}{K}} X_k^j$$

$$X_k^j \xrightarrow{d_x} 0$$

While we already implement a cost of infection in reproduction, we assume that infection does not influence the competition outcome nor intrinsic death for the sake of simplicity. However, this cost of death can be easily implemented if necessary.

Possible events in the parasite population

Z^k is the free-living parasite population size of the genotype k. ${Q_x}_k^j \in [0,1]$ is the probability of successful infection of a parasite with genotype k to prey with genotype j, which depends on the allelic states of the interacting prey-parasite genotypes. $S \in R^+$ is the encounter rate between prey and free-living parasites. When a parasite successfully infects the prey, the parasite is removed from the free-living parasite population. Otherwise, it remains in the free-living parasite population. We note here that the parasite does not reproduce in the prey. As it is computationally expensive to simulate single prey individuals carrying multiple parasite genotypes, we assume that the free-living parasites only infect uninfected prey.

$$X_0^j + Z^k \xrightarrow{{Q_x}_k^j * S} X_k^j$$

$$X_0^j + Z^k \xrightarrow{(1-{Q_x}_k^j) * S} X_0^j + Z^k$$

All parasite individuals have an intrinsic death rate $d_z \in R^+$, leading to the ultimate extinction of the parasite if there are no predator and prey species.

$$Z^k \overset{d_z}{\rightarrow} 0$$

Possible events in predator population

Y_z^l is the predator population size of the genotype l. When $z = 0$, the predator does not carry a parasite. Let us introduce the notation Y_0^c, where $c = l$ when the new offspring is the same genotype as the parent, and $c \neq l$ when the new offspring is a mutant. The probability of no mutation happening during reproduction is $(1-\mu_y)^n$, where $\mu_y \in [0,1]$ is the probability of a mutation per locus and n is the number of loci in the genotype. The predation rate per individual encounter between a prey and a predator is f_y. The reproduction efficiency of the predator by consuming a prey is $k_y \in [0,1]$. We note here that the predator offspring is parasite-free, Y_0^c. When an uninfected prey meets an uninfected predator, we have the following reactions.

$$Y_0^l + X_0^j \xrightarrow{f_y * k_y} Y_0^l + Y_0^c$$

$$Y_0^l + X_0^j \xrightarrow{f_y * (1-k_y)} Y_0^c$$

When a prey of genotype j, infected with a parasite of genotype k meets an uninfected predator of genotype l, the predator can be infected with probability ${Q_y}_k^l \in [0,1]$. If the predator is successfully infected by the parasite through the consumption of infected prey, the infected predator's ability to reproduce is affected by the presence of the parasite and hence is suppressed according to the parameter $r_p \in [0,1]$. Meanwhile, the parasite transmitted from the primary host, the prey, will reproduce within the definitive host, the predator, and have $nZ^b \in Z^+$ offspring per successful infection. The original parasite stays in the predator and later dies together with the predator stochastically. The parasite offspring, nZ^b, are released to begin a new life cycle, where $b = k$ when the new offspring is the same genotype as the parent, and $b \neq k$ when the new offspring is a mutant. The probability of no mutation happening during reproduction is $(1-\mu_z)^n$, where $\mu_z \in [0,1]$ is the probability of a mutation per locus and n is the number of loci in the genotype.

$$Y_0^l + X_k^j \xrightarrow{f_y * r_p * k_y * {Q_y}_k^l} Y_k^l + Y_0^c + nZ^b$$

$$Y_0^l + X_k^j \xrightarrow{f_y * (1-r_p * k_y) * {Q_y}_k^l} Y_k^l + nZ^b$$

If the parasite is not successful in infecting the predator through trophic transmission, the prey and parasite, X_k^j, die. In this case, there is still a suppression of predator reproduction due to exposure $r_e \in [0,1]$. We assume that the reproduction suppression due to infection is larger than the one due to exposure. Hence, $r_p < r_e \leq 1$.

$$Y_0^l + X_k^j \xrightarrow{f_y * r_e * ky * \left(1 - Q_{y_k}^l\right)} Y_0^l + Y_0^c$$

$$Y_0^l + X_k^j \xrightarrow{f_y * \left(1 - r_e * k_y\right) * \left(1 - Q_{y_k}^l\right)} Y_0^l$$

When an infected predator Y_z^l (where z refers to any parasite genotype) consumes a parasite-free prey of genotype j, the infected predator can reproduce at the cost r_p. Note parasite reproduction already happened when this predator was first time infected, thus is not redundantly included here.

$$Y_z^l + X_0^j \xrightarrow{f_y * r_p * k_y} Y_z^l + Y_0^c$$

$$Y_z^l + X_0^j \xrightarrow{f_y * \left(1 - r_p * k_y\right)} Y_z^l$$

Finally, we consider the remaining possible reactions where an infected prey meets an infected predator. When an infected predator Y_z^l consumes a prey of genotype j infected with a parasite of genotype k, the predator can produce offspring without the parasite Y_0^c. If the infection fails, the prey and parasite, X_k^j, die. We apply the suppression of predator reproduction as before by parameter r_e.

$$Y_z^l + X_k^j \xrightarrow{f_y * r_e * r_p * k_y * \left(1 - Q_{y_k}^l\right)} Y_z^l + Y_0^c$$

$$Y_z^l + X_k^j \xrightarrow{f_y * \left(1 - r_e * r_p * k_y\right) * \left(1 - Q_{y_k}^l\right)} Y_z^l$$

If the infected predator is successfully infected again by the newly encountered infected prey, its ability to reproduce is further suppressed by the same parameter $\mathrm{r_p}$. The parasite transmitted by this newly encountered prey will reproduce in its final host because this is the first time this predator is infected by this individual parasite. As it is computationally expensive to simulate multiple genotypes in single predator individuals, and we do not need to keep track of those genotypes as they are no longer transferred to other hosts, we simply use the notation Y_z^l to refer to the infected predator after a new infection, where z refers to any parasite genotype.

$$Y_z^l + X_k^j \xrightarrow{f_y * {r_p}^2 * k_y * Q_{y_k}^l} Y_z^l + Y_0^c + nZ^b$$

$$Y_z^l + X_k^j \xrightarrow{f_y*(1-{r_p}^2*k_y)*{Q_y}_k^l} Y_z^l + nZ^b$$

All predator individuals have an intrinsic death rate $d_y \in R^+$, leading to the ultimate extinction of the predator if there are no prey species. While we already implement a cost of infection in reproduction, we assume that infection does not influence intrinsic death for the sake of simplicity. However, this cost of death can be easily implemented if necessary.

$$Y_z^l \xrightarrow{d_y} 0$$

Model Simulations

We ran multiple independent realisations using the Gillespie algorithm (Gillespie, 1977; Pineda-Krch, 2008) to investigate how parasite-mediated selection impacts our coevolving system. We used different combinations of the parameters r_x and r_p, to capture the parasite-mediated selection on the prey and the predator, respectively. Note that the parameters r_x and r_p range between 0 and 1, with an increment of 0.1, i.e., r_x = 0, 0.1, 0.2, …, 1, and r_p = 0, 0.1, 0.2, …, 1, where 0 indicates the parasite completely suppresses the host's ability to reproduce, and a value of 1 that there is no parasite impact on the host reproduction (see details in the individual-based model). We ran 100 independent realisations for each combination of r_x and r_p. In every realisation, we used the same initial population sizes, i.e., 800 prey individuals, 1,000 parasite individuals, and 100 predator individuals (see choice of the initial population sizes in Supplementary Note 1 and choice of the number of realisations in Supplementary Note 3).

Data Analyses

To obtain the likelihood of species coexistence, we performed 100 independent realisations for each parameter combination r_x and r_p. We recorded the number of realisations in which the predator, prey, or parasite went extinct.

The abundance and genotypic diversity of each species were recorded in single time points when the three species coexisted, for each parameter combination of r_x and r_p. To account for the abundance and genotypic diversity in the prey and predator populations, we considered infected and non-infected individuals. To account for the

abundance and genotypic diversity in the parasite population, we considered the free-living individuals. To avoid the over-representation of initial states where the species have not co-evolved away from the initial conditions, we used the average of 100 realisations from time point 200-1000 to represent the abundance and genotypic diversity in each parameter combination of r_x and r_p. Supplementary Note 3 explains the choice of the time points for averages of abundance and genotypic diversity.

We performed a linear model to explore the correlation between the abundance and genotypic diversity of the predator. We conducted quadratic regressions to explore the correlations between the abundance and genotypic diversity of the prey and the parasite. We additionally conducted quadratic regressions to explore the correlations between the abundance and genotypic lifespan, as well as between the genotypic diversity and genotypic lifespan, of the three entities. We refer to genotypic lifespan as the number of time steps from the moment a genotype emerged until it went extinct. To avoid the noise due to continuously arising mutations with low frequencies, we focused on the effective genotypes making up at least 2% of the population.

Finally, for the likelihood of the emergence of supertypes, we recorded the number of realisations in which a supertype emerged when the three species coexisted.

References

Anaya-Rojas, J. M., Best, R. J., Brunner, F. S., Eizaguirre, C., Leal, M. C., Melián, C. J., Seehausen, O., & Matthews, B. (2019). An experimental test of how parasites of predators can influence trophic cascades and ecosystem functioning. *Ecology*, *100*(8), 1–12. https://doi.org/10.1002/ecy.2744

Brockhurst, M. A., Buckling, A., & Rainey, P. B. (2006). Spatial heterogeneity and the stability of host-parasite coexistence. *Journal of Evolutionary Biology*, *19*(2), 374–379. https://doi.org/10.1111/j.1420-9101.2005.01026.x

Brunner, F. S., Anaya-Rojas, J. M., Matthews, B., & Eizaguirre, C. (2017). Experimental evidence that parasites drive eco-evolutionary feedbacks. *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.1619147114

Buckingham, L. J., & Ashby, B. (2022). Coevolutionary theory of hosts and parasites. *Journal of Evolutionary Biology*, *December 2021*, 1–20. https://doi.org/10.1111/jeb.13981

Campbell, A. (1961). Conditions for the existence of Bacteriophage. *Evolution*, *15*(2), 153–165.

De Castro, F., & Bolker, B. (2005). Mechanisms of disease-induced extinction. *Ecology Letters*, *8*(1), 117–126. https://doi.org/10.1111/j.1461-0248.2004.00693.x

De Meester, L., Vanoverbeke, J., Kilsdonk, L. J., & Urban, M. C. (2016). Evolving Perspectives on Monopolization and Priority Effects. *Trends in Ecology and Evolution*, *31*(2), 136–146. https://doi.org/10.1016/j.tree.2015.12.009

Eizaguirre, C., Lenz, T. L., Kalbe, M., & Milinski, M. (2012). Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. *Nature Communications*, *3*, 1–6. https://doi.org/10.1038/ncomms1632

Frickel, J., Theodosiou, L., & Becks, L. (2017). *Rapid evolution of hosts begets species diversity at the cost of intraspecific diversity*. https://doi.org/10.1073/pnas.1701845114

Gillespie, D. T. (1977). Exact Stochastic Simulation of Coupled Chemical Reactions. *The Journal of Physical Chemistry*, *81*(25), 2340–2361.

Gotanda, K. M., Delaire, L. C., Raeymaekers, J. A. M., Pérez-Jvostov, F., Dargent, F., Bentzen, P., Scott, M. E., Fussmann, G. F., & Hendry, A. P. (2013). Adding parasites to the guppy-predation story: Insights from field surveys. *Oecologia*, *172*(1), 155–166. https://doi.org/10.1007/s00442-012-2485-7

Govaert, L., Fronhofer, E. A., Lion, S., Eizaguirre, C., Bonte, D., Egas, M., Hendry, A. P., De Brito Martins, A., Melián, C. J., Raeymaekers, J. A. M., Ratikainen, I. I., Saether, B., Schweitzer, J. A., & Matthews, B. (2019). Eco-evolutionary feedbacks—Theoretical models and perspectives. *Functional Ecology*, *33*, 13–30. https://doi.org/10.1111/1365-2435.13241

Hairston Jr., N. G., Ellner, S. P., Geber, M. A., Yoshida, T., & Fox, J. A. (2005). Rapid evolution and the convergence of ecological and evolutionary time. *Ecology Letters*, *8*, 1114–1127. https://doi.org/10.1111/j.1461-0248.2005.00812.x

Heilmann, S., Sneppen, K., & Krishna, S. (2010). Sustainability of Virulence in a Phage-Bacterial Ecosystem. *Journal of Virology*, *84*(6), 3016–3022. https://doi.org/10.1128/jvi.02326-09

Heilmann, S., Sneppen, K., & Krishna, S. (2012). Coexistence of phage and bacteria on the boundary of self-organized refuges. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(31), 12828–12833. https://doi.org/10.1073/pnas.1200771109

Híjar-Islas, A. C., Milne, A., Eizaguirre, C., & Huang, W. (2023). Parasite-mediated predation determines the infection in a complex predator-prey system. bioRxiv doi: 10.1101/2023.10.29.564596

Huang, W., Hauert, C., & Traulsen, A. (2015). Stochastic game dynamics under demographic fluctuations. *PNAS*, *112*. https://doi.org/10.1073/pnas.1418745112

Huang, W., Werner, B., & Traulsen, A. (2012). The impact of random frequency-dependent mutations on the average population fitness. *BMC Evolutionary Biology*, *12*(1). https://doi.org/10.1186/1471-2148-12-160

Kuris, A. M., Hechinger, R. F., Shaw, J. C., Whitney, K. L., Aguirre-Macedo, L., Boch, C. A., Dobson, A. P., Dunham, E. J., Fredensborg, B. L., Huspeni, T. C., Lorda, J., Mababa, L., Mancini, F. T., Mora, A. B., Pickering, M., Talhouk, N. L., Torchin, M. E., & Lafferty, K. D. (2008). Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature*, *454*(7203), 515–518. https://doi.org/10.1038/nature06970

Lafferty, K. D., Arim, M., Briggs, J., Leo, G. De, Dobson, P., Dunne, J. A., Johnson, P. T. J., Armand, M., Pablo, A., & Pascual, M. (2008). *Parasites in food webs: the ultimate missing links*. 533–546. https://doi.org/10.1111/j.1461-0248.2008.01174.x

Lafferty, K. D., & Kuris, A. M. (2002). Trophic strategies, animal diversity and body size. *TRENDS in Ecology and Evolution*, *17*(11), 507–513. https://doi.org/10.1080/10428190410001683697

Lennon, J. T., & Martiny, J. B. H. (2008). Rapid evolution buffers ecosystem impacts of viruses in a microbial food web. *Ecology Letters*, *11*, 1178–1188. https://doi.org/10.1111/j.1461-0248.2008.01225.x

Lion, S., Van Baalen, M., & Wilson, W. G. (2006). The evolution of parasite manipulation of host dispersal. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1590), 1063–1071. https://doi.org/10.1098/rspb.2005.3412

Melián, C. J., Vilas, C., Baldó, F., González-Ortegón, E., Drake, P., & Williams, R. J. (2011). Eco-evolutionary dynamics of individual-based food webs. In *Advances*

in Ecological Research (Vol. 45, Issue October). https://doi.org/10.1016/B978-0-12-386475-8.00006-X

Mougi, A., & Iwasa, Y. (2010). Evolution towards oscillation or stability in a predator-prey system. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1697), 3163–3171. https://doi.org/10.1098/rspb.2010.0691

Pineda-Krch, M. (2008). GillespieSSA: Implementing the stochastic simulation algorithm in R. *Journal of Statistical Software*, *25*(12), 1–18. https://doi.org/10.18637/jss.v025.i12

Retel, C., Kowallik, V., Huang, W., Werner, B., Künzel, S., Becks, L., & Feulner, P. G. D. (2019). The feedback between selection and demography shapes genomic diversity during coevolution. *Science Advances*, *5*(10). https://doi.org/10.1126/sciadv.aax0530

Retel, C., Märkle, H., Becks, L., & Feulnet, P. G. D. (2019). Ecological and Evolutionary Processes Shaping Viral Genetic Diversity. *Viruses*, *11*, 1–16. https://doi.org/10.3390/v11030220

Romero-Mujalli, D., Jeltsch, F., & Tiedemann, R. (2019). Individual-based modeling of eco-evolutionary dynamics: state of the art and future directions. *Regional Environmental Change*, *19*(1), 1–12. https://doi.org/10.1007/s10113-018-1406-7

Rudolf, V. H. W., & Antonovics, J. (2005). Species coexistence and pathogens with frequency-dependent transmission. *American Naturalist*, *166*(1), 112–118. https://doi.org/10.1086/430674

Sasaki, A. (2000). Host-parasite coevolution in a multilocus gene-for-gene system. *Proceedings of the Royal Society B: Biological Sciences*, *267*(1458), 2183–2188. https://doi.org/10.1098/rspb.2000.1267

Schmid-Hempel, P. (2021). *Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics*. Oxford University Press.

Tellier, A., Moreno-Gámez, S., & Stephan, W. (2014). Speed of adaptation and genomic footprints of host-parasite coevolution under arms race and trench warfare dynamics. *Evolution*, *68*(8), 2211–2224. https://doi.org/10.1111/evo.12427

Thompson, J. N., & Burdon, J. J. (1992). Gene-for-gene coevolution between plants and parasites. *Nature*, *360*, 121–125.

Yoshida, T., Jones, L. E., Ellner, S. P., Fussmann, G. F., & Hairston Jr., N. G. (2003). Rapid evolution drives ecological dynamics in a predator-prey system. *Nature*, *424*, 303–306.