

Quantitative host resistance drives the evolution of increased virulence in an emerging pathogen

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Abstract

Emergent infectious diseases can have a devastating impact on host populations. The high selective pressures on both the hosts and the pathogens frequently lead to rapid adaptations not only in pathogen virulence but also host resistance following an initial outbreak. However, it is often unclear whether hosts will evolve to avoid infection-associated fitness costs by preventing the establishment of infection (here referred to as *qualitative resistance*) or by limiting its deleterious effects through immune functioning (here referred to as *quantitative resistance*). Equally, the evolutionary repercussions these different resistance mechanisms have for the pathogen are often unknown. Here, we investigate the co-evolutionary dynamics of pathogen virulence and host resistance following the epizootic outbreak of the highly pathogenic bacterium *Mycoplasma gallisepticum* in North American house finches (*Haemorrhous mexicanus*). Using an evolutionary modelling approach and with a specific emphasis on the evolved resistance trait, we demonstrate that the rapid increase in the frequency of resistant birds following the outbreak is indicative of strong selection pressure to reduce infection-associated mortality. This, in turn, created the ecological conditions that selected for increased bacterial virulence. Our results thus suggest that quantitative host resistance was the key factor underlying the evolutionary interactions in this natural host–pathogen system.

Introduction

Antagonistic interactions between hosts and pathogens can give rise to intense selection pressures and trigger rapid evolutionary changes in both (Buckling & Rainey, 2002; Paterson *et al.*, 2010). This is particularly true in the context of novel disease outbreaks, in which potentially devastating impacts on the host population are expected to feed back to the pathogen through a rapidly changing host environment (Lively, 1989; Best & Kerr, 2000; Paterson *et al.*, 2010). When faced with high infection-associated fitness costs, hosts can evolve either to prevent the establishment of infection, referred to as *qualitative resistance*, or limit its deleterious effects through immune function, referred to as

quantitative resistance (Gandon & Michalakis, 2000). Note that within the context of this work, quantitative resistance can be understood as an umbrella term that also includes a notion of *tolerance*, if the latter simply refers to any host defence that limits infection virulence. Although evolution of different resistance strategies has been observed in many species, the distinction between qualitative and quantitative resistance in animal populations is rarely made. Understanding such mechanisms is important, however, in particular for predicting the likely direction of virulence evolution.

There is a large body of literature using theoretical models to investigate the effect of host resistance on pathogen exploitation strategy and the subsequent evolution of virulence (see e.g. Regoes *et al.*, 2000; Miller *et al.*, 2006; Carval & Ferriere, 2010; Best *et al.*, 2014). Based on evolutionary theory, studies have suggested, for example, that a high proportion of qualitatively resistant hosts, that is hosts that are less susceptible to

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an infection, will limit transmission opportunities by decreasing pathogen prevalence and thus select for lower pathogen virulence (van Baalen, 1998; Gandon & Michalakis, 2000). On the other hand, quantitative resistance traits that permit infections but decrease infection duration, for example through immune activation, are expected to select for higher virulence (van Baalen, 1998; Gandon & Michalakis, 2000; Gandon *et al.*, 2002). With the notable exception of the well-characterized introduction of *Myxoma* virus in European rabbit populations, which resulted in patterns of rapid, reciprocal host–pathogen adaptation (Fenner & Chapple, 1965; Best & Kerr, 2000; Kerr & McFadden, 2002; Stanford *et al.*, 2007), compelling empirical evidence for such co-evolutionary interactions in wild, as opposed to laboratory populations, is still limited (Janzen, 1980; Bonneaud *et al.*, 2018).

The outbreak of the bacterium *Mycoplasma gallisepticum* in North American house finches (*Heamorrhous mexicanus*) mid-1990s following a jump from poultry provides us with a unique opportunity to disentangle the evolutionary interplay between host resistance and pathogen virulence in a natural system. The ensuing epizootic of severe conjunctivitis led to the death of millions of house finches (Hochachka & Dhondt, 2000; Kollias *et al.*, 2004), as a result of increased predation or blindness associated starvation (Ley *et al.*, 1996; Fischer *et al.*, 1997; Hartup *et al.*, 1998; Roberts *et al.*, 2001). In the following years, the rapid evolution of host resistance was observed, with the frequency of resistant individuals rising from ~20% to ~80% within 12 years following the outbreak (Bonneaud *et al.*, 2011; Adelman *et al.*, 2013). Finches from disease-exposed and disease-unexposed populations were indeed initially shown to display equivalent gene expression responses to experimental infection with *M. gallisepticum*, which then subsequently diverged as genetic resistance spread in the former (Bonneaud *et al.*, 2011; Bonneaud *et al.*, 2012b). The evolution of resistance was independently verified in a recent time-shift experiment involving 56 *M. gallisepticum* isolates sampled over the 20 years of the epizootic from outbreak, which allowed a demonstration of host–pathogen coevolution in this system with host resistance adaptively driving increased *M. gallisepticum* virulence over time (Bonneaud *et al.*, 2018). Although hosts from disease-exposed populations were found to have evolved the ability to mount a protective cell-mediated immune response (Bonneaud *et al.*, 2012b), whether resistance has evolved only to protect from infection-induced morbidity or mortality, or whether it has also evolved to prevent infection establishment, and what is the contribution of either type of resistance mechanism, remains to be clarified. In addition, which type of resistance mechanism is likely to have driven the evolution of increased pathogen virulence also remains to be established.

Here, we used a modelling approach to study the *M. gallisepticum* – house finch disease system and investigate the likely mechanism of host resistance that led to host and pathogen evolution. Building on previous findings of rapid resistance evolution, this approach enabled us to apply hypotheses generated in previous theoretical studies (Gandon & Michalakis, 2000) to an important and well-characterized avian system. Our results suggest that the observed spread of host resistance was the result of strong selection pressure to reduce *M. gallisepticum* induced mortality, which in turn provided the competitive advantage for more virulent bacteria to take hold in the population.

Materials and methods

In order to investigate the evolutionary dynamics of host resistance and pathogen virulence, we developed a two-strain, two-phenotype SIRS model with seasonal forcing. We divided the host population, N (which is not assumed to remain constant), into two broad categories, resistant (N_r) and nonresistant (N_{nr}) hosts, with resistant hosts assumed to carry a resistance trait offering lower susceptibility to infection, faster infection clearance rate or lower disease-associated mortality. Hosts can become infected with either a high virulence strain (h) or a low virulence strain (l) of *M. gallisepticum* and transmission is frequency dependent. For simplicity, we assumed that upon recovery birds gain full but waning immunity against reinfection. Interactions between strains, such as partial cross-immunity or super-infection, were not considered.

The rate of change in the number of susceptible ($S_{nr,r}$), infected ($I_{nr,r}^{h,l}$) and recovered birds ($R_{nr,r}$) was given by the following set of differential equations:

$$\frac{dS_{nr}}{dt} = b(t)N_{nr} - \beta(t)((I_r^l + I_{nr}^l) + \lambda_t(I_r^h + I_{nr}^h))\frac{S_{nr}}{N} + \delta R_{nr} - \mu(t)S_{nr}$$

$$\frac{dS_r}{dt} = b(t)N_r - \rho_s\beta(t)((I_r^l + I_{nr}^l) + \lambda_t(I_r^h + I_{nr}^h))\frac{S_r}{N} + \delta R_r - \mu(t)S_r$$

$$\frac{dI_{nr}^l}{dt} = \beta(t)(I_r^l + I_{nr}^l)\frac{S_{nr}}{N} - \sigma I_{nr}^l - \zeta I_{nr}^l - \mu(t)I_{nr}^l$$

$$\frac{dI_{nr}^h}{dt} = \lambda_t\beta(t)(I_r^h + I_{nr}^h)\frac{S_{nr}}{N} - \lambda_r\sigma I_{nr}^h - \lambda_m\zeta I_{nr}^h - \mu(t)I_{nr}^h$$

$$\frac{dI_r^l}{dt} = \rho_s\beta(t)(I_r^l + I_{nr}^l)\frac{S_r}{N} - \rho_r\sigma I_r^l - \rho_m\zeta I_r^l - \mu(t)I_r^l$$

$$\frac{dI_r^h}{dt} = \rho_s \lambda_t \beta(t) (I_r^h + I_{nr}^h) \frac{S_r}{N} - \rho_r \lambda_r \sigma I_r^h - \rho_m \lambda_m \zeta I_r^h - \mu(t) I_r^h$$

$$\frac{dR_{nr}}{dt} = \sigma (I_{nr}^l + \lambda_r I_{nr}^h) - \delta R_{nr} - \mu(t) R_{nr}$$

$$\frac{dR_r}{dt} = \rho_r \sigma (I_r^l + \lambda_r I_r^h) - \delta R_r - \mu(t) R_r$$

where $b(t)$ is the seasonal birth rate, $\beta(t)$ is the seasonal transmission coefficient, σ is the recovery rate, ζ is the disease-associated mortality rate and $\mu(t)$ is the natural and season-dependent death rate. Figure S1 illustrates this model as a flow diagram.

We used two sets of scaling factors to investigate the (independent) effect(s) of host resistance (ρ_s, ρ_r, ρ_m) and pathogen virulence ($\lambda_t, \lambda_r, \lambda_m$), where the subscripts denote the affected traits (s : susceptibility, t : transmissibility, r : recovery, m : mortality). For example, ρ_m describes the relative decrease in infection-associated mortality (quantitative resistance) for birds of the resistant phenotype, whereas λ_t describes the relative increase in the transmission rate of birds infected with the more virulent strain, representing the relationship between increased virulence and transmissibility (Alizon *et al.*, 2009). Considering host susceptibility, recovery rate and mortality rate independently allow us to investigate the full spectrum of resistance, from *qualitative* ($\rho_s < 1, \rho_r, \rho_m = 1$, i.e. reduced susceptibility) to *quantitative* ($\rho_s = 1, \rho_r \geq 1, \rho_m \leq 1$, i.e. increased clearance and/or decreased mortality). Note, for simplicity we did not consider any of the resistance traits to affect transmissibility *per se*.

Seasonal changes in house finch demography and aggregation rates have previously been shown to be important in generating semi-annual cycles of *M. gallisepticum* prevalence (Altizer *et al.*, 2004; Hosseini *et al.*, 2004). We therefore incorporated seasonality into our model by using time-dependent birth, death and transmission rates derived from previous studies. Birth rates, $b(t)$, peak in July/August, when chicks fledge after breeding pair formation and nesting earlier in the year (Hill, 1993; Altizer *et al.*, 2004), whereas mortality, $\mu(t)$, is highest during winter months, as house finches are known to be more susceptible to cold stress, potentially influencing over-winter survival, particularly in Northern and Mid-Western states of the United States (Dawson *et al.*, 1983). Driven by social aggregation during the mating season and the formation of winter foraging flocks, we assumed that transmission rates, $\beta(t)$, fluctuate biannually (Altizer *et al.*, 2004). The seasonal birth, mortality and transmission rates are given as follows:

$$b(t) = b_0 (\sin((t - 0.1)\pi))^{k_b}$$

$$\mu(t) = \mu_0 (0.4 + 0.6 \sin((t - 0.5)\pi))^{k_d}$$

$$\beta(t) = \beta_0 (0.2 + 0.8 (\sin((t - 0.15)\pi)^{k_t} + \sin((t + 0.4)\pi)^{k_t}))$$

with b_0 , μ_0 and β_0 denoting the peak birth, death and transmission rates, respectively. k_b , k_d and k_t are (even-valued) shape parameters that determine the length of a season. Under default parameter settings (Table 1), this model generates the observed biannual dynamics with two distinct peaks in spring and autumn corresponding to seasonal increases in host population densities and aggregation and hence transmission opportunities (Altizer *et al.*, 2004; Hosseini *et al.*, 2004) (Figs S2 and S3). Note, as the birth and death rates are independent and since we consider the possibility of infection-induced mortality, the total population size $N(t)$ is not constant over time.

We initialized the model assuming a 20% background prevalence in host resistance (in line with empirical observations (Bonneaud *et al.*, 2011)) and an initially low prevalence of the more virulent strain ($I^h(0) = 0.05 I^l(0)$). The mean length of infection was set to 2 months ($\sigma = 6/\text{year}$) and the loss of immunity was set to $\delta = 0.9/\text{year}$, based on estimating average recovery and waning immunity from previous experimental infections of wild-caught finches (Kollias *et al.*, 2004). Baseline infection-associated mortality was set at ten times the natural death rate ($\zeta = 3$), which resulted in a ~40% reduction

Table 1 Model parameters and parameter ranges. Defaults values are based on qualitative model fit to observed data, whereas their respective ranges, as considered in the model sensitivity analyses, are defined to lie within the confines of the values that are biologically reasonable.

Parameter	Description	Value	Range
σ	Recovery rate	6 year ⁻¹	[5, 10]
δ	Rate of loss of immunity	0.9 year ⁻¹	[0.5, 1.5]
ζ	Disease-associated death rate	3 year ⁻¹	[2, 4]
k_t	Shape parameter (transmission)	20	–
k_b	Shape parameter (birth)	80	–
k_d	Shape parameter (death)	10	–
β_0	Max transmission coefficient	48 year ⁻¹	–
b_0	Max birth rate	3.61 year ⁻¹	–
μ_0	Background mortality rate	0.3 year ⁻¹	–
ρ_s	Susceptibility scale factor (resistance)	1	[0, 1]
ρ_r	Recovery scale factor (resistance)	1	[1, 2]
ρ_m	Mortality scale factor (resistance)	0.1	[0, 1]
λ_t	Transmissibility scale factor (virulence)	1.2	[1, 2]
λ_r	Recovery scale factor (virulence)	1	[0.5, 1]
λ_m	Mortality scale factor (virulence)	2	[1, 5]

in population density at epizootic emergence, in line with empirical data on density-dependent declines at this time (Hochachka & Dhondt, 2000). Under baseline settings, we assumed that the high virulent strain (HV) is more transmissible than the low-virulent strain (LV) that is $\lambda_t > 1$, but that this transmission advantage is offset by higher mortality rates (i.e. $\lambda_m > 1$), such that its overall fitness is lower. Although energetic costs of resistance to *M. gallisepticum* have been demonstrated in experimental infections of resistant populations (Bonneaud *et al.*, 2012a), we decided to exclude resistance-associated costs from our model as rapid population-level spread of resistance indicates that the fitness benefits of resistance would significantly outweigh such costs. Table 1 provides a summary of the model's parameters.

Due to the large number of free parameters in our model, we ran full sensitivity analyses based on Latin Hypercube sampling using 3000 random (and uniformly distributed) samples of nine parameters (the six scaling parameters plus the rates describing recovery, loss of immunity and disease-associated mortality) within the ranges shown in Table 1. The measures of interest, for example the proportion of resistant hosts and the number of infected individuals, were then smoothed using either Gaussian kernel density estimation (for single-parameter sensitivity analyses using the *Gaussian_kde* function from the Python *scipy.stats* module) or using a Gaussian Process regression model (for two-parameter sensitivity using the *GPR* function from the GPy Python package). The parameters describing the seasonality in birth, death and transmission were derived by qualitatively fitting our model to empirical data (Fig. S3) and then kept constant throughout.

In all cases, we ran our model using the following initial conditions:

$$S_{nr} = 7900, S_r = 1990, I_{nr}^l = 20, I_{nr}^h = 1, I_r^l = 5, \\ I_{nr}^h = 0.25, R_{nr} = 0, R_r = 0$$

Results

General model behaviour assuming quantitative resistance

We first simulated our model under default parameter settings considering all hosts as equally susceptible to becoming infected, but with resistant and nonresistant hosts differing in their infection-induced mortality rates ($\rho_s = 1, \rho_r = 1, \rho_m = 0.1$). In line with empirical observations, we found that the number of susceptible hosts and the total host population size decreased significantly following the initial outbreak as a direct result of high infection-associated mortality rates (Fig. 1a). This decrease in the number of susceptible hosts was followed by a substantial decline in disease prevalence (Fig. 1b). When we stratified the host

population into resistant and nonresistant phenotypes (Fig. 1c), our model revealed rapid phenotypic changes in the host population in line with previous empirical studies (Bonneaud *et al.*, 2011), with resistant hosts reaching ~90% prevalence after around 12 years post-emergence. In parallel, although the low virulence pathogen strain dominated during the initial phase of the epizootic, it became outcompeted by the more virulent strain after around nine years (Fig. 1b,d). What these results suggest is that strong pathogen-induced selection pressure and the subsequent increase in resistant host phenotypes in the population created the conditions for a more virulent pathogen strain to emerge and dominate.

We next considered qualitative resistance as a result of an increased rate of parasite clearance (i.e. $\rho_r > 1$). Although in this case we could also observe selection for host resistance, this occurred at a much reduced rate and resulted in a significant reduction in either parasite prevalence or population size (Fig. S4). Overall, we found that the model behaviour with regard to host phenotypic change and general epidemiological dynamics was far less sensitive to changes in the relative increase in parasite clearance, ρ_r , than to changes in disease-associated mortality, ρ_m (Fig. S5). For this reason, we decided to concentrate predominantly on the latter for further analysis.

Waning immunity has a strong effect on the spread of resistance

Before going into the details about the most likely resistance trait underlying the observed shift in host phenotypes and selection for more virulent bacteria, we examined the model's sensitivity with regard to changes in the recovery rate, σ , and the rate at which birds lose immunity against reinfection, δ . The duration of infection in house finches in experimental settings can vary between 1 and 4 months, whereas from experimental reinfection of pre-exposed finches, it was found that finches could still mount protective immune responses that reduced infection severity up to 14 months after their first exposure (Sydenstricker *et al.*, 2005). However, some degree of variation between individuals and populations is expected. To account for these uncertainties, we ran our sensitivity analysis (see Methods) over wide ranges of values for the recovery rate, σ ($\sigma \in [5, 10]$), and loss of immunity, δ ($\delta \in [0.5, 1.5]$) (Fig. S6). This showed that waning immunity (δ) has a much stronger effect on the disease and selection dynamics, with higher rates of immunity loss leading to a higher turnover in the susceptible population, which in turn maintains higher disease prevalence and selection pressure for host resistance. The recovery rate only had a comparatively small effect, at least within the ranges considered here, whereby longer infection periods increase disease prevalence and

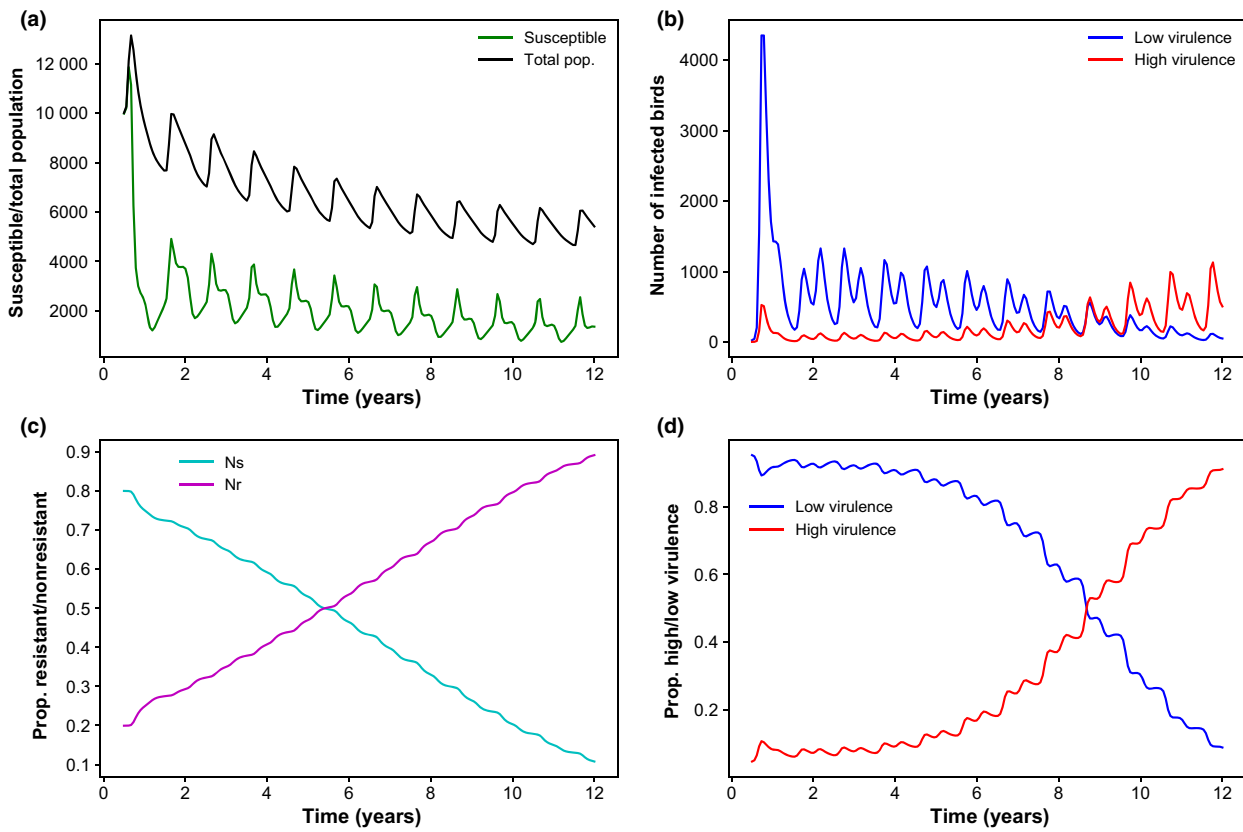


Fig. 1 Model simulations of host and pathogen population dynamics. (a) Following disease emergence, there is a significant decrease in the number of susceptible hosts (S , green line) and total host population size (N , black line). (b) Number of individuals infected with the low virulence ($I_{nr}^l + I_r^l$, blue line) and high virulence strain ($I_{nr}^h + I_r^h$, red line) over time showing initial dominance of the low-virulent strain, which eventually gets outcompeted. (c) Change in the proportion of the host population with a nonresistant or resistant phenotype (N_{nr} and N_r , respectively). (d) Proportion of host population infected with low or high virulence strains over time showing how the high virulence strain gains a competitive advantage due to the higher number of resistant individuals in the population. Parameter values as in Table 1.

the rate at which resistance spreads through the host population.

Qualitative resistance reduces the speed of phenotypic change

As shown in Fig. 1, quantitative resistance by means of reducing disease-associated mortality (ρ_m) can cause a rapid change in host phenotype distribution, with resistant birds increasing in frequency from ~20% to ~80% in just ten years, in line with empirical observations. We next examined the comparative effect of qualitative resistance by reducing susceptibility to infection (ρ_1) instead. We thus mapped the population size and the proportion of resistant birds at 12 years post-emergence against the considered parameter ranges of ρ_s (relative susceptibility of resistant hosts) and ρ_m (relative mortality of resistant hosts) as a result of our sensitivity analysis over the entire parameter space (see Methods). As shown in Fig. 2a,b, both have similar effects on the long-term trajectory of the population but a decrease in

infection susceptibility naturally causes a reduction in overall infection prevalence and hence selection pressure for host resistance. As a result, the rate of host phenotypic change is markedly slowed under the assumption of qualitative resistance, as shown in Fig. 2c,d. In fact, what Fig. 2c suggests is that the observed increase in resistant birds in the population within such a short period of time is mostly compatible with a marked reduction in infection-induced mortality. This implies that although some reduction in susceptibility (qualitative resistance) cannot be ruled out, the epidemiological dynamics and rapid spread of host resistance in the *M. gallisepticum* – house finch disease system – have most likely been driven by quantitative resistance that limits the mortality of infected birds.

Host resistance and its effect on virulence evolution

Next, we considered the selective impact of host resistance on the evolution of pathogen virulence. As demonstrated above, quantitative resistance as a

decrease in mortality (ρ_m) was found to induce a rapid change in host phenotypes and was further associated with the selection of more virulent bacteria over time. Furthermore, our results implied that the degree of susceptibility must be similar between resistant and non-resistant hosts in order to maintain high disease prevalence levels and the associated selection pressure (Fig. 2). Also, as indicated in Fig. 1, the change in host phenotype frequency in the population appeared to create the condition for a more virulent bacterial strain to emerge and become dominant. We thus examined the effect of both qualitative and quantitative resistance on disease prevalence and the evolution towards higher bacterial virulence.

From Fig. 3a,b, it is clear that reducing infection-induced mortality has a much stronger and positive

effect on disease prevalence than decreasing susceptibility. Moreover, the higher selection pressure as a result of quantitative resistance is also much more likely to provide a competitive advantage of the more virulent strain. This is demonstrated in Fig. 3c, showing the relative prevalence of the high virulent strain (HV) under changes in ρ_s and ρ_m at 12 years post-emergence. Under default parameter settings, we find that the high virulent strain dominates only when infection-associated mortality is low (Fig. 3d, top graph), whereas under qualitative resistance disease prevalence is generally low and the less virulent strain persists (Fig. 3d, bottom graph).

However, the exact conditions that favour a more virulent strain crucially depend, amongst other things, on its mortality rate relative to that of a less virulent

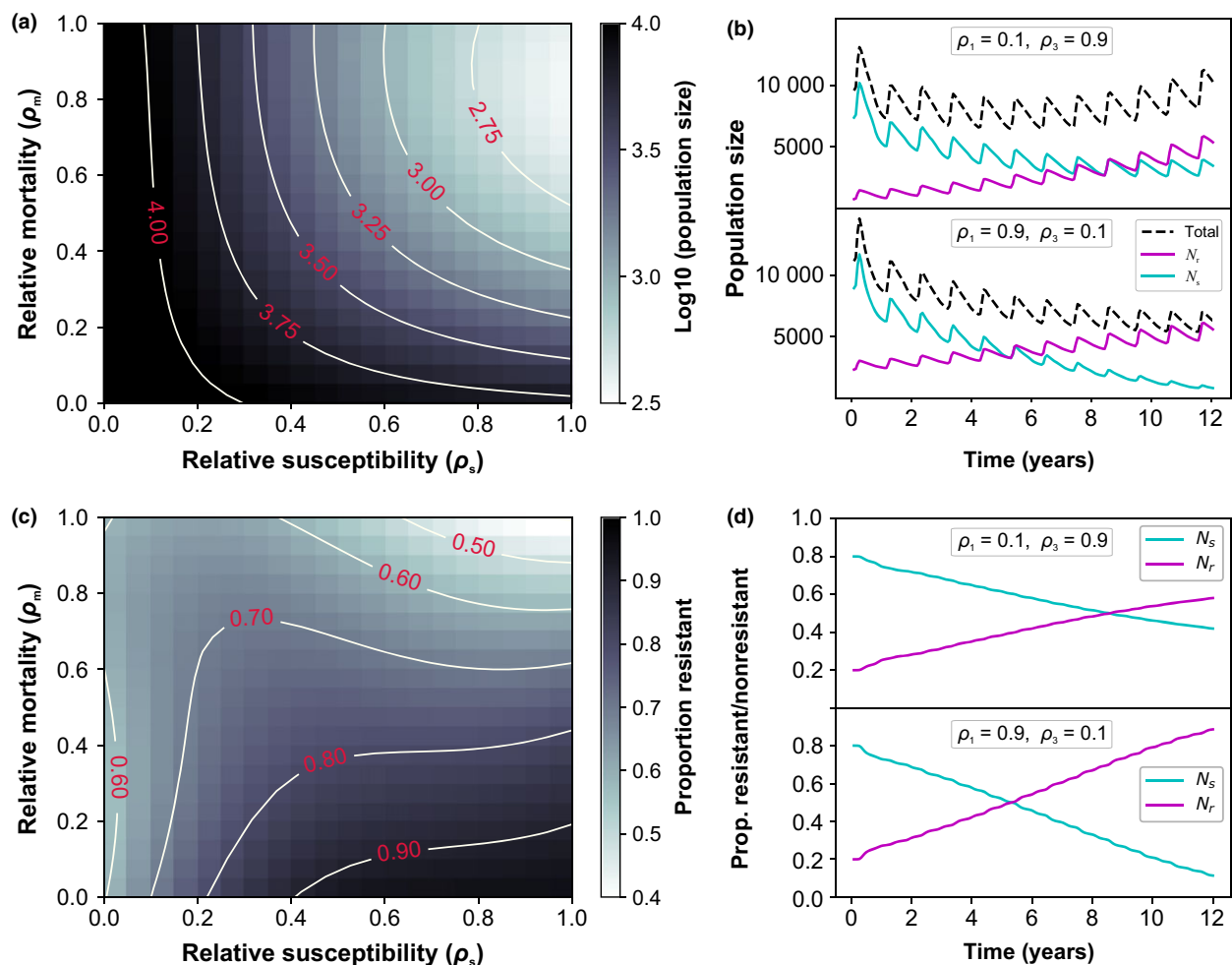


Fig. 2 The effect of host resistance traits on the spread of host resistance. (a, c) Host population size and proportion of resistant hosts at 12 years post-emergence mapped against relative mortality (ρ_m) and relative susceptibility (ρ_s) and based on Latin hypercube sensitivity analyses over the entire parameter space. (b, d) Example time series of the number of resistant and non-resistant hosts for two dominant resistance traits (quantitative resistance, top graphs, and qualitative resistance, bottom graphs) showing how selection for host resistant is much faster under the assumption of quantitative resistance. Parameter values as in Table 1 unless indicated.

strain, λ_m , and the extent to which infection-induced mortality is reduced in resistant hosts, ρ_m . That is, in the absence of (a sufficiently high number of) resistant hosts in the population, the assumed transmission advantage of the more virulent strain was offset by excess host mortality, such that its overall fitness is lower than a less virulent strain. To demonstrate how changes in host phenotype, or more specifically the rise in resistance against infection-induced mortality, can shift the balance in favour of higher virulence, we examined the model's sensitivity to those two scaling factors (λ_m and ρ_m) and recorded the relative frequency of the high- and low-virulent strain and total infection prevalence.

As shown in Fig. 4a, under the assumption that disease-associated mortality of the more virulent strain (λ_m) is high, selection will favour the less virulent strain as the gain in transmissibility will be outweighed by the rapid loss of infected hosts. However, with an increase in host resistance against disease-induced mortality (i.e. reducing ρ_m), overall infection prevalence increases (Fig. 4b) and more virulent strains are able to dominate (Fig. 4a). A similar behaviour can also be observed when instead of increasing transmissibility; the more virulent strain gains a transmission advantage through longer infectious periods (i.e. decreasing λ_r , shown in Fig. S7). Note, at this point we only considered higher virulence as an increase in infection-associated mortality without a potentially beneficial effect of increasing transmissibility. We therefore also examined the scenario where transmissibility and mortality are coupled. As expected and illustrated in Fig. 4c, increasing transmission by means of higher virulence can quickly offset the fitness cost of excess mortality, leading to much wider parameter region where a more virulent bacteria can emerge and competitively outcompete a less virulent strain.

Discussion

In the present study, we identified the set of conditions that could explain the rapid increase in host resistance and pathogen virulence following the epizootic outbreak of *M. gallisepticum* in North American house finches. Specifically, we have demonstrated that neither a reduction in host susceptibility to the establishment of *M. gallisepticum* infection, nor an increase in parasite clearance rates alone, is compatible with the empirical data. Indeed, the impact of each alone on infection prevalence would cause a reduction in disease-induced selection pressure to the extent that we would no longer be able to observe shifts in both host and pathogen phenotypes. Instead, our results suggest that the rapid, disease-induced selection of host resistance traits based on reducing infection-associated mortality must accompany either reduced susceptibility or recovery period, for instance through a lowering of pathogen

load, for a subsequent increase in bacterial virulence in this important host–pathogen system.

Our results are in line with both empirical observations and theoretical predictions. Experimental work on the evolution of resistance in house finches has shown that resistance spread rapidly from standing genetic variation in <12 years of disease exposure (Bonneaud *et al.*, 2011). This speed of host adaptation suggests that the disease must have imposed a strong selection pressure on the host population, a hypothesis further supported by the high rates of mortality observed in the wild following outbreak (Hochachka & Dhondt, 2000). In accordance, our modelling study demonstrates that for a phenotypic change to occur in the host population, infection with *M. gallisepticum* must incur a high fitness cost on house finches (in terms of increased mortality), with nonresistant finches paying a much higher cost than resistant ones.

The results of our model further suggest that resistant finches not only experience reduced mortality when infected, but that they should also display a level of susceptibility to infection similar to that of nonresistant birds. When host resistance is modelled as reduced susceptibility to infection, its negative impact on population-level infection prevalence is indeed too great to maintain the selection pressure that would account for the rapid change in host phenotype frequencies. A similar outcome was obtained when modelling resistance as increased recovery rate: selection pressure on host resistance subsequently dropped, thereby slowing down the speed of host phenotypic change. The most likely resistance trait under selection in this system therefore consists of a reduction in infection severity (potentially through a reduction in pathogen load), which is compatible with the notion of quantitative resistance.

It has previously been proposed that qualitative resistance, which lowers a host's susceptibility to infection establishment, should select for decreased pathogen virulence and that this effect should positively increase with the proportion of resistant individuals in the host population (Gandon & Michalakis, 2000). Although we also found some conditions under which such reduced susceptibility could lead to an increase in virulence, the parameter regions where this occurred resulted in model behaviours that are incompatible with the observed data and potentially lead to either (host or pathogen) population extinction or a reduction in selection pressure precluding major shifts in host phenotypes.

A number of studies to date have focussed on characterizing the immune response of wild populations to emerging infectious diseases (Kerr & McFadden, 2002; Gregory *et al.*, 2005; Bonneaud *et al.*, 2012b). The canonical example of the parallel evolution of host resistance and pathogen virulence following disease emergence is the eradication attempt of European rabbits (*Oryctolagus cuniculus*) of Australia using the *Myxoma*

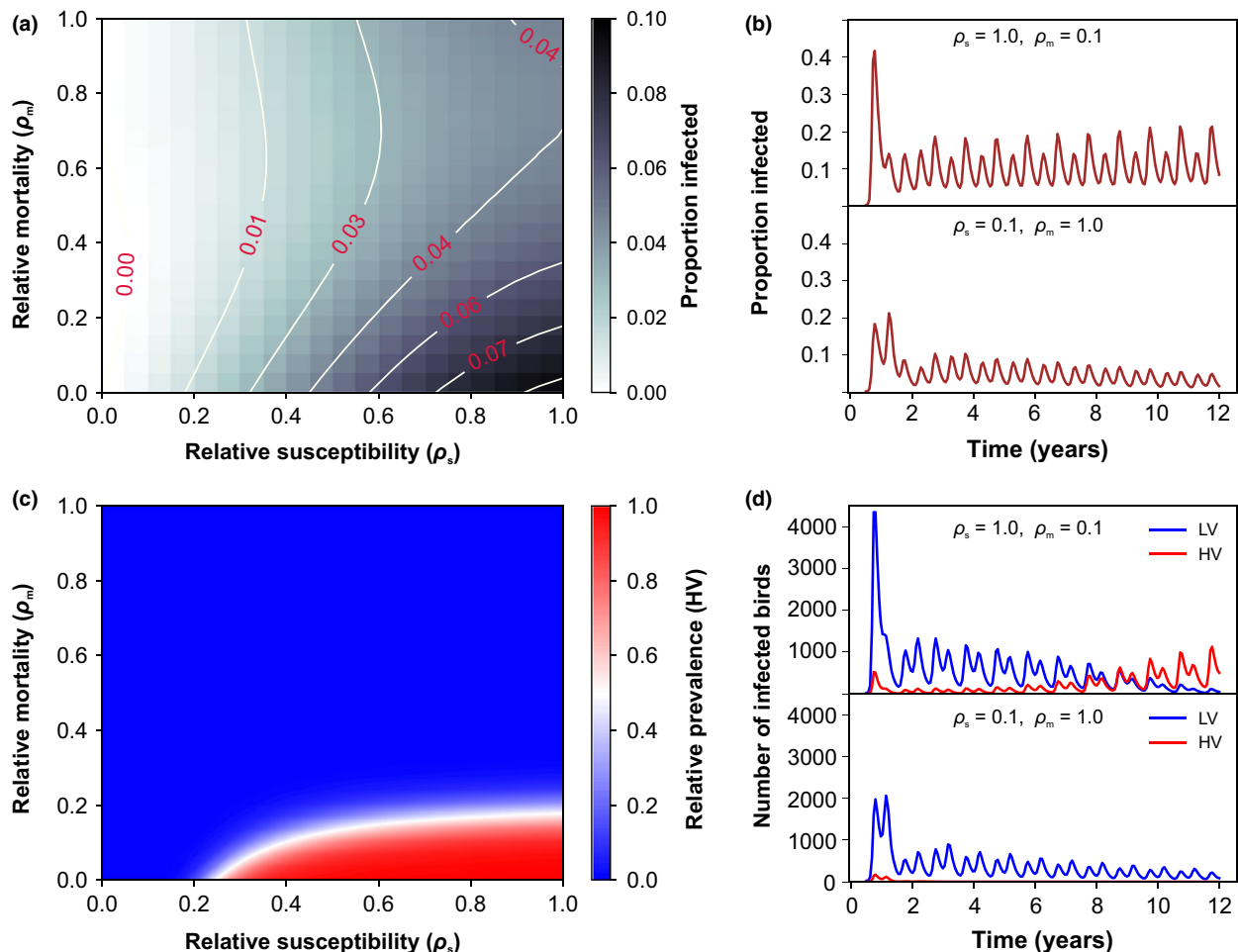


Fig. 3 Effects of host resistance on pathogen virulence evolution. (a) Proportion of infected hosts at 12 years post-emergence mapped against relative mortality (ρ_m) and relative susceptibility (ρ_s) and based on Latin hypercube sensitivity analyses over the entire parameter space showing how a reduction in susceptibility can significantly decrease disease prevalence in the population. (b) Example time series of the proportion of infected birds under quantitative resistance (reduction in mortality, top graph) or qualitative resistance (reduced susceptibility, bottom graph). (c) Relative prevalence of the high virulent strain (HV) at 12 years post-emergence under default parameter settings (Table 1). Only in situations where relative mortality is much reduced can the more virulent strain become dominant. (d) Example time series showing the number of birds infected with either the low or high virulent bacteria (blue line and red line, respectively).

virus. Following the release of highly virulent strains in 1950, which resulted in a dramatic decline of the rabbit population by over 99% (Marshall *et al.*, 1955), virulence was found to decrease and resistance via enhanced innate immunity to spread in the host population (Best & Kerr, 2000). Although these findings suggest that quantitative resistance may have driven the evolution of resistance in this case, genes underlying both qualitative and quantitative forms of resistance can be found in the wild, suggesting a role of both in shaping host–pathogen interactions and coevolution. For example, a study on wild great tits showed that different supertypes of the same MHC gene can confer either qualitative or quantitative resistance to avian malaria (Sepil *et al.*, 2013), suggesting that the

distinction between qualitative and quantitative forms of resistance is useful not only in plant systems, but also in wild animal populations where it is scarcely applied. The form of resistance (qualitative or quantitative) under selection is likely to impact phenotypic change expected in the host population, as well as the pathogen's evolutionary trajectory.

Our model suggests that a decrease in infection-associated mortality is the most important component in resistance that could select for the host/pathogen co-evolutionary patterns observed in this disease system. However, as our model shows only qualitative patterns and does not invoke specific mechanisms of pathogen clearance, results from the model alone could either be interpreted as resistance through immune activation

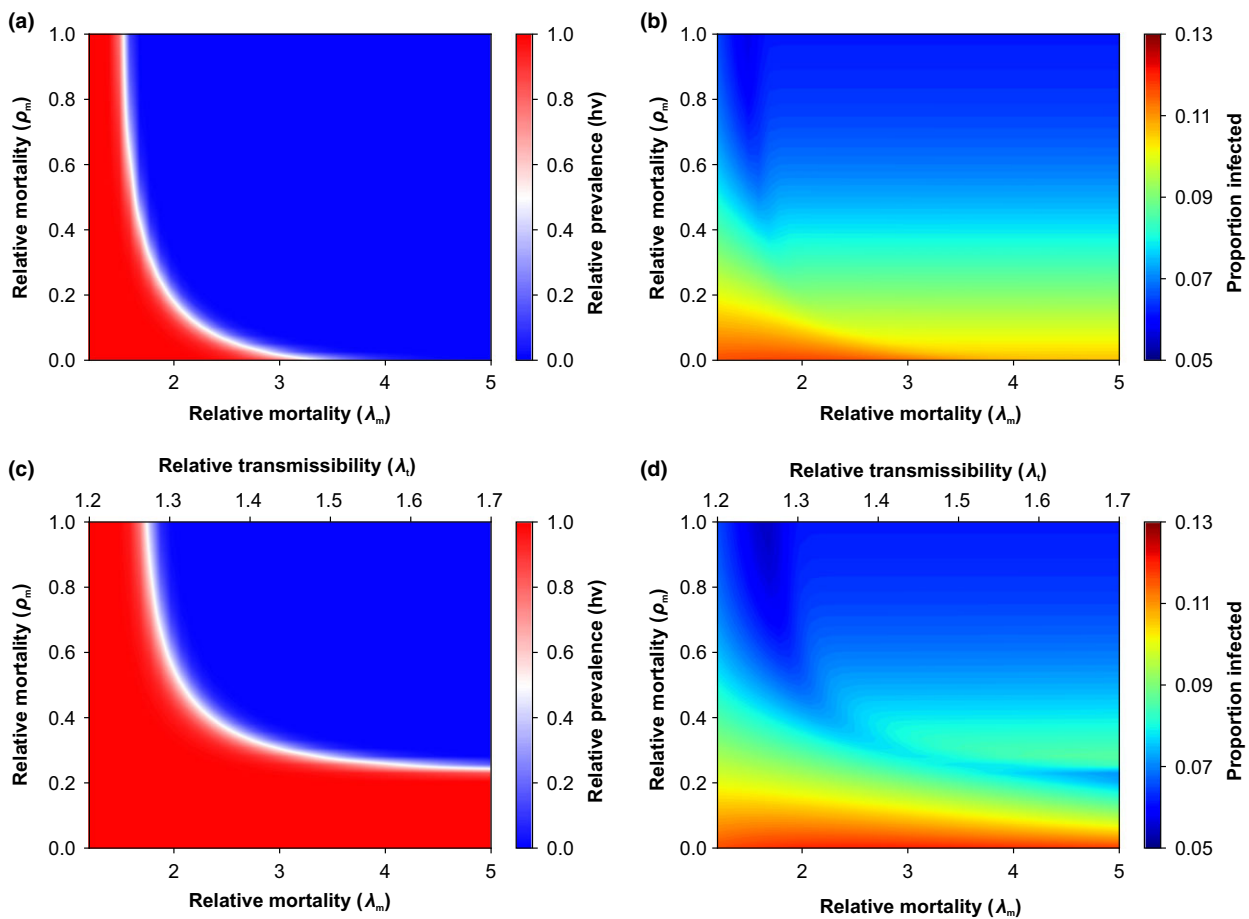


Fig. 4 The effect of resistance-reduced mortality (ρ_m) and virulence-induced mortality (λ_m) on virulence evolution and infection prevalence. (a) Relative prevalence of the high virulence strain (HV) at 12 years post-emergence shows how a more virulent strain can only gain a competitive advantage when offset by a marked reduction in infection-induced mortality in resistant hosts. (b, d) The proportion of the host population infected at 12 years post-emergence. Virulence-induced mortality (λ_m) only has a small effect on population-wide infection levels, whereas host resistance against infection-associated mortality (λ_m) strongly influences disease prevalence. (c) Relative prevalence of the high virulence strain (HV) assuming virulence-associated transmissibility and mortality is coupled, leading to an increase in the parameter space where the higher virulent bacteria have the competitive advantage. Unless stated otherwise, parameters as in Table 1.

that reduced mortality, or as mortality tolerance, whereby hosts simply live longer with infection, thus increasing the infectious period of the pathogen (Best *et al.*, 2008). Evidence for the evolution of resistance in this system comes from two independent experimental infection studies of house finches from disease-exposed and disease-unexposed populations with multiple bacterial isolates varying in virulence (Bonneaud *et al.*, 2018). Soon after outbreak, finches from disease-exposed and disease-unexposed populations displayed equivalent gene expression responses to *M. gallisepticum* infection that were consistent with pathogen-induced immune suppression (Bonneaud *et al.*, 2011). These responses, however, were shown to subsequently diverge as hosts from disease-exposed populations

evolved genetic resistance and the ability to mount protective cell-mediated immune responses, which resulted in lower pathogen load at the site of infection (Bonneaud *et al.*, 2011, 2012b, 2018). On the other hand, the previous suggestion for the evolution of tolerance in this system is based on a lack of differences in pathogen load between hosts from disease-exposed and disease-unexposed populations, which is likely to have resulted from inoculation with a nonvirulent bacterial isolate (Adelman *et al.*, 2013). Although it is possible that tolerance mechanisms limiting pathogen-induced immune manipulation may have accompanied the evolution of host resistance in this system (Staley & Bonneaud, 2015), protective immune processes that reduce mortality, rather than mortality tolerance, are therefore

likely to have driven the evolution of pathogen virulence that we detected in our study.

An important aspect influencing the long-term evolutionary outcome of host–pathogen interactions is the costs associated with either form of resistance. It has been proposed that resistance through protective immunity is expected to evolve only when the cost of mounting the immune response is lower than the cost of being infected (Antonovics & Thrall, 1994; Boots & Bowers, 2004; Viney *et al.*, 2005). House finches from populations that evolved resistance have previously been found to lose more body mass following experimental infections with *M. gallisepticum* than finches from unexposed populations (Bonneaud *et al.*, 2012a). The fact that resistance has spread despite this indicates that the fitness benefit of resisting infection ultimately outweighs the shorter-term energetic cost of resistance. Hence, although our model does not include costs associated with resistance, we do not expect such costs to impact the results of this study other than by influencing the probability that resistance will go to fixation and that resistant phenotypes will decline in frequency once the disease goes extinct.

The results of our model are fairly robust to changes in parameter values and thus allow for differing estimates and uncertainties, such as in the recovery period, mortality rates and rate of waning immunity based on field studies (Altizer *et al.*, 2004) compared to experimental infections (Kollias *et al.*, 2004; Sydenstricker *et al.*, 2005). It is also worth noting that disease dynamics vary geographically (Hosseini *et al.*, 2004) and potentially encompass wide confidence intervals. The parameter regions in which we can create dynamics compatible with the data are therefore likely wider than reported here, although the general conclusions with regard to the actual trait most likely being responsible for the observed dynamics would still apply.

Our results show that the duration of immunity (δ) strongly affects disease prevalence and selection pressure by directly regulating the turnover in the susceptible population. This is in concordance with recent findings by (Fleming-Davies *et al.*, 2018), who suggest that increases in the duration of an incomplete form of immunity gives a selective advantage to virulent *M. gallisepticum* strains during secondary infections. Although the same immunity that protects hosts from damage can also drive parallel increases in bacterial virulence, we argue that the selective consequence of resistance during primary infections is the more parsimonious explanation for virulence evolution in this system.

In conclusion, our results reiterate the important influence of the mechanisms underlying host resistance on the mode and tempo of host phenotypic change and pathogen virulence. Furthermore, as the varying impacts of different forms of resistance on the evolution of pathogen virulence, this might also have consequences for disease control measures as inappropriate

intervention can potentially result in undesirable outcomes (see e.g. Gandon *et al.*, 2001; Stevens *et al.*, 2007). Although our results are specific to this particular disease system, they do highlight the general need for a more detailed investigation of host–pathogen interactions not only to understand co-evolutionary dynamics and but also to minimize adverse effects of disease control.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Flow diagram of the 2 strain - 2 phenotype SIR model.

Figure S2 Seasonal variation in birth, death and disease transmission.

Figure S3 Short-term dynamics of population level *M. gallisepticum* infection prevalence.

Figure S4 The effect of parasite clearance on host and pathogen population dynamics.

Figure S5 A sensitivity analysis of the effect of (ρ_m) vs. ρ_r on population size, rate of host evolution and infection prevalence.

Figure S6 The effect of the recovery rate and the loss of immunity on selection on resistance and infection prevalence.

Figure S7 Effect of relative mortality and recovery on pathogen evolution and prevalence.

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