Evolutionary Ecology Research, 2018, **19**: 257–278

The evolutionary response of virulence to host heterogeneity: a general model with application to myxomatosis in rabbits co-infected with intestinal helminths

Morgan P. Kain[1], Isabella M. Cattadori[2,3] and Benjamin M. Bolker[1,4]

[1]*Department of Biology, McMaster University, Hamilton, Ontario, Canada,* [2]*Center for Infectious Disease Dynamics, Pennsylvania State University, University Park, Pennsylvania, USA,* [3]*Department of Biology, Pennsylvania State University, University Park, Pennsylvania, USA and* [4]*Department of Mathematics and Statistics, McMaster University, Hamilton, Ontario, Canada*

ABSTRACT

Background: Changes in the mean and variance of traits in a host population modify selection pressures on pathogen virulence; increasing heterogeneity (variance) leads to the over- or under-exploitation of a subset of hosts and thus decreases the pathogen's ability to spread in the population.

Objective: To improve the links between theory and data, we develop a model of pathogen evolution in heterogeneous host populations that can be parameterized with a range of empirical measures of host heterogeneity to infection, and which is sufficiently flexible to capture the life history of many natural host–pathogen systems. We use this model to determine whether rabbits co-infected with gastrointestinal helminths could have contributed to the attenuation of the myxoma virus, and might explain differences in the virulence of strains more recently circulating in Australia and Scotland.

Methods: We constructed a deterministic model of pathogen transmission and solved it numerically to determine evolutionarily stable strategies with respect to transmission and virulence. Using this model and empirical data from the rabbit–myxoma virus system, we examine how host heterogeneity in co-infection with gastrointestinal helminths affects the severity of an evolved pathogen in a given host type, and to what degree host heterogeneity affects a pathogen's ability to spread in the host population.

Results: Host heterogeneity to infection always decreases pathogen spread and often leads to the under-exploitation of a typical host. However, the specifics are sensitive to: the shape of the distribution describing host heterogeneity, the relationship between virulence and transmission, and the relationship between the heterogeneous host trait and pathogen virulence. In the rabbit–myxoma virus system, gastrointestinal helminths plausibly contributed to the attenuation of the myxoma virus but are unlikely to have contributed to the higher virulence of circulating strains in Scotland relative to those in Australia in the years following the release of the virus.

Keywords: evolutionarily stable strategy, MYXV, trade-off, vector-borne.

Correspondence: M.P. Kain, Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada. email: kainm@mcmaster.ca *or* morganpkain@gmail.com

INTRODUCTION

Heterogeneity among hosts to infection influences host–pathogen interactions and, in doing so, pathogen evolution. For example, host heterogeneity can affect the evolution of pathogen virulence, defined broadly as the harm caused by pathogens to hosts, and thus the severity of a disease at the host population level. Host heterogeneity may select for lower virulence (Ebert and Hamilton, 1996; Gandon, 2004; Pugliese, 2011; Osnas and Dobson, 2012), for higher virulence (Gandon *et al.*, 2001; Ganusov *et al.*, 2002; Read *et al.*, 2015), or facilitate the co-existence of two strains with different exploitation strategies (Fleming-Davies *et al.*, 2015). Pathogen evolution in a heterogeneous host population can also disproportionately harm a subset of the population, where disease severity can increase in the most susceptible hosts, such as unvaccinated individuals (Gandon *et al.*, 2001). Moreover, host heterogeneity can decrease a pathogen's absolute fitness at its evolutionary optimum. For example, heterogeneity can reduce the intrinsic reproduction number (R_0) of a pathogen that cannot adjust its exploitation strategy to its current host (Regoes *et al.*, 2000; Gandon, 2004).

Previous theoretical studies have clarified many of the intricacies generated by the interactions among host heterogeneity to infection, host–pathogen dynamics, and evolution. However, the complexity of the models used, and the variation in model structures and assumptions across studies, make it difficult to calibrate models from empirical data and to predict outcomes for the pathogen and for hosts. Improving the link between theory and empirical data is a critical step in advancing our understanding of pathogen evolution – arguably the most important challenge currently faced in research on the ecology and evolution of infectious diseases (Alizon and Michalakis, 2015; Cressler *et al.*, 2016). While the field has made substantial progress, for example by parameterizing virulence–transmission trade-off curves for a range of pathogens (Fraser *et al.*, 2007; De Roode *et al.*, 2008; Doumayrou *et al.*, 2013; Berngruber *et al.*, 2015), and by quantifying the relationship between pathogen virulence and transmission across the full life cycle of the pathogen (De Roode *et al.*, 2008; Kain and Bolker, 2017), connections between theory and data that account for the role of host heterogeneity in pathogen evolution remain rare.

Here we present a model for the evolution of pathogen virulence in a heterogeneous host population that is flexible enough to be parameterized with a range of empirical measures of host heterogeneity (such as host age, immune status, genotype, vaccine status, or co-infection with other parasitic species), and which is sufficiently tractable to capture the life history of many natural host–pathogen systems. We parameterize our model with empirical data from the *Oryctolagus cuniculus*–myxoma virus (MYXV) system, the canonical example of host–pathogen evolution following pathogen invasion (Fenner and Marshall, 1957; Dwyer *et al.*, 1990; Fenner and Fantini, 1999). We examine variation among rabbits in gastrointestinal helminth burden, which is known to interact with the outcome of MYXV infection (Cattadori *et al.*, 2007). The effects of co-infection by multiple pathogen species on the evolution of virulence have been previously examined (Choisy and de Roode, 2010; Restif and Graham, 2015); however, despite the important role of co-infection in infection and transmission dynamics (Cattadori *et al.*, 2007; Graham *et al.*, 2007; Fenton, 2008; Thakar *et al.*, 2012; Cattadori *et al.*, 2014), the effects of heterogeneity in co-infection on the evolution of a focal pathogen have rarely been considered. We explore the impact of a heterogeneous helminth burden on the severity of an evolved pathogen in a given host type and on the pathogen's ability to spread in the host population. We also evaluate whether or not the observed differences in the virulence of circulating MYXV strains in Scotland and Australia can plausibly be explained by differences in the prevalence of gastrointestinal helminths.

METHODS

Model structure

Our model examines the evolutionarily stable strategy (ESS) of a pathogen exploiting a heterogeneous host population. We define ESS virulence as the virulence that maximizes a pathogen's intrinsic reproductive number R_0, the expected number of new infections a single infected individual generates in an otherwise susceptible population [this criterion, derived from a more general criterion of non-invasibility, holds for a broad range of epidemiological models (Alizon *et al.*, 2009)]. Given that we assume a relationship between transmission rate and virulence (Alizon *et al.*, 2009; Cressler *et al.*, 2016), ESS virulence determines both overall pathogen severity at the population level and pathogen severity in a subset of the population, and indirectly affects the pathogen's ability to spread. Virulence is not fundamentally an intrinsic trait of the pathogen, but rather the result of a complex interaction between host and pathogen. Virulence can be defined as the disease-induced mortality rate of a host within a homogeneous population, or as the mortality rate of an infected *reference host* in a heterogeneous population. In the *O. cuniculus*–MYXV system, the virulence of a circulating MYXV strain is defined as the mortality rate of an infected laboratory breed of rabbit with no shared evolutionary history with the virus (Fenner and Marshall, 1957). In a heterogeneous host population, infection with a single pathogen strain will cause a range of mortality rates. For consistency with the *O. cuniculus*–MYXV system literature, we define ESS virulence in the heterogeneous population as the disease-induced mortality rate of a reference rabbit with no helminths. Alternatively, the reference host could be defined as a well-nourished host, a fully immunocompetent or immunodeficient host, or a host with a reference genotype.

Our model comprises three flexible components: (1) a trade-off curve that relates pathogen virulence to transmission (Alizon *et al.*, 2009); (2) the distribution of *observable heterogeneity* in the host population, which we modelled as a continuous distribution, in contrast to some previous studies that used discrete groups; and (3) the *heterogeneity map*, i.e. a function that translates observable heterogeneity to the impacts on the host interaction with the pathogen, such as host mortality rate. Mathematically, the observable heterogeneity distribution and the heterogeneity map are redundant – we need only the distribution of pathogen-induced host mortality or pathogen clearance rate to predict ESS virulence. However, we include both components in our model to facilitate empirical parameterization and testing of the model. Once the heterogeneity map has been parameterized using laboratory experiments or field data, for example, only the observable heterogeneity, such as secondary infection severity among hosts, is required to determine pathogen ESS virulence. Ultimately, this framework should allow easier model calibration and prediction of ESS virulence in a variety of systems.

Trade-off curve

Due to the trade-off between virulence and transmission (Anderson and May, 1982; Ewald, 1983), transmission (β) is assumed to be an increasing function of virulence (α). When β is a decelerating function of α, a single value of α maximizes R_0 (Alizon *et al.*, 2009). We examine both power-law and sigmoidal functions for the relationship between α and β; these functions are commonly used to model directly transmitted and vector-borne diseases, respectively (Alizon and van Baalen, 2005). Both transmission rate (β) and instantaneous rate of

disease-induced host mortality (virulence, α) can be modelled as a function of within-host pathogen exploitation strategy [e.g. replication rate, titre, or set-point viral load (Fraser *et al.*, 2007)], though this relationship does not need to be explicitly modelled and can be ignored without loss of generality. Here we model pathogen exploitation strategy (σ) as titre load and assume a linear relationship between φ and α ($\alpha = b\varphi$), which has previously been assumed in the *O. cuniculus*–MYXV system (Anderson and May, 1982; Dwyer *et al.*, 1990).

A power-law relationship is given by:

$$\beta(\alpha) = c\alpha^{\frac{1}{\gamma}},$$

with $\alpha = b\varphi$, where b is the slope of the linear relationship between replication rate and mortality rate. For the remainder of the paper we focus on α, using $b = 0.1$ in all simulations. The shape of the curve is controlled by γ (larger γ increases curvature and decreases optimal virulence), while c is a scaling factor. The value for R_0 is given by $(\beta(\alpha))/(\mu + \alpha)$, or for the power law:

$$R_0 = \frac{c\alpha^{\frac{1}{\gamma}}}{\mu + \alpha},$$

where μ is the background rate of host mortality. In this case, the α that maximizes R_0 is given by

$$\alpha^* = \frac{\mu}{\gamma - 1}.$$

When the trade-off curve follows a sigmoidal curve, for example the Hill function (Tjørve, 2003),

$$\beta(\alpha) = \frac{c\alpha^n}{\alpha + \alpha^n},$$

then

$$R_0 = \frac{c(b\alpha)^n}{(a + (b\alpha)^n)(\mu + b\alpha)},$$

where n and a jointly control the location of the inflection point and slope of the curve at the inflection point, and c is a scaling parameter. The α that optimizes R_0 for the Hill function has no closed-form solution but can be found numerically. Figure 1 shows examples of a power-law and sigmoidal trade-off curve.

Host heterogeneity

We assume hosts possess a single trait that determines their reaction to pathogen infection (e.g. host age, immune status, genotype, vaccine status, or co-infection with another pathogen species). We use a Gamma distribution to model the among-host variation (a continuous analog of the negative binomial distribution more commonly used to quantify macro-parasite load), exploring a range of scenarios by adjusting the Gamma shape and scale parameters. Unlike Gandon (2004), we assume that all pathogen strains are perfectly implastic and can only adopt a single strategy across all hosts. This assumption is supported

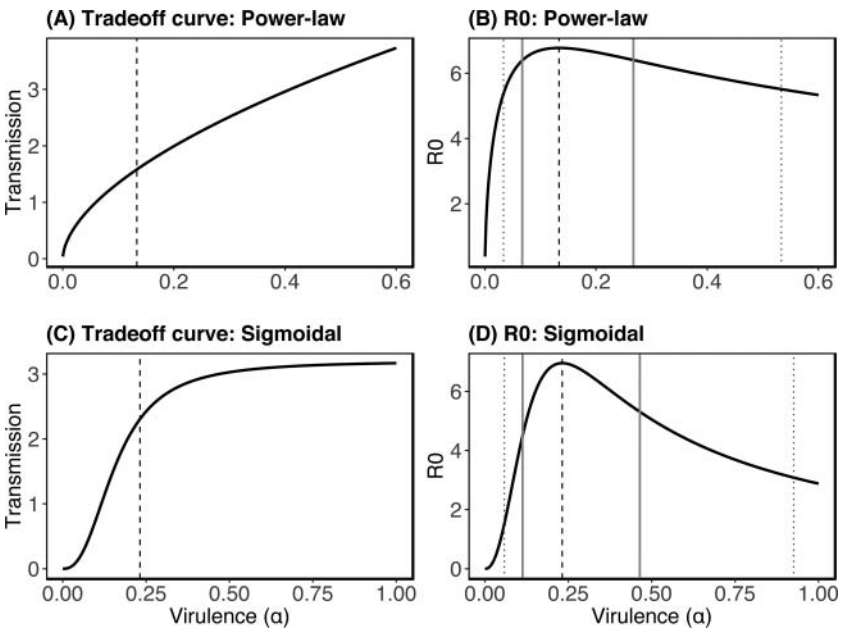

Fig. 1. Panels A and B show an example of a power-law trade-off curve and the R_0 curve that results from this power-law trade-off curve, respectively. Panels C and D show an example of a sigmoidal trade-off curve and the R_0 curve that results from this sigmoidal trade-off curve, respectively. The vertical dashed black lines show optimum α values. The vertical dotted black lines and solid grey lines show the R_0 for a 2× and 4× higher or lower α than optimum, respectively. The parameter values used here were specifically chosen to accentuate the differences between the power-law and sigmoidal trade-off curves (power-law: $c = 5$, $\gamma = 1.75$, $m = 0.2$, $\mu = 0.1$; sigmoidal: $a = 0.01$, $b = 0.1$, $n = 2.5$, $\mu = 0.01$, $c = 3.2$). Figure S1 shows power-law and sigmoidal trade-off curves with the parameter values used in the simulation for Figs. 2–4 (with $\gamma = 1.05$ for the power-law relationship and $c = 1.5$ for the sigmoidal relationship).

for the *O. cuniculus*–MYXV system (Best and Kerr, 2000; Kerr *et al.*, 2017); however, evidence for pathogen plasticity is evident in other systems [e.g. *P. aeruginosa* infection in mammals (Furukawa *et al.*, 2006)].

Heterogeneity map

The distribution of host heterogeneity is translated into a distribution of virulence using a heterogeneity map. Our first choice of heterogeneity function describes a monomolecular, or saturating exponential, relationship between a host trait and pathogen virulence:

$$\alpha_h = \alpha(z(1 - e^{-rx}) + 1),$$

where z controls the maximum of the curve, r controls the rate of approach to z, and α_h is the mortality rate of a host with a trait value (e.g. helminth burden) equal to x. A saturating function is a plausible first approximation of the relationship between a host trait, such as nutrient status or immunocompetence, and pathogen virulence (Bedhomme *et al.*, 2004). In

the MYXV–helminth case, this function describes the increasing, but saturating effect of helminth burden on MYXV virulence, reflecting a type-1/type-2 immune response trade-off (for details, see 'The *O. cuniculus*–myxoma virus case study', p. 264).

Alternatively, we consider a heterogeneity map that allows for a reduction in pathogen virulence at intermediate values of the heterogeneous trait. To model this situation, we use a piecewise function that combines a quadratic function with the saturating exponential function. This function could be used, for example, to capture variation in the immune response, where a smaller immune upregulation reduces pathogen virulence but a larger upregulation increases virulence because of an increase in pathogen exploitation in an effort to evade the stronger immune constraints or immunopathology. For the *O. cuniculus*–myxoma virus case study (see below), we use this piecewise function to model a primed immune response, where low to intermediate helminth burdens reduce MYXV virulence.

$$\alpha_h = \begin{cases} \left(\dfrac{x}{h} - 1\right)^2 (1-k) + k, & x \le h \\ z(1 - e^{-r(x-h)}) + k, & x > h \end{cases}$$

Here h determines the location of minimum pathogen virulence, which defines the helminth burden that provides a rabbit with the largest decrease in MYXV virulence (i.e. the lowest possible MYXV mortality rate: α_h). This function starts at 1 and decreases to a helminth burden of h following a quadratic relationship, where relative virulence at h is given by k ($0 < k < 1$). At trait values greater than h, virulence increases to $z + 1$ following a saturating exponential function. The complete model is illustrated in Fig. 2.

Model outcomes

From a host-centric view, this model can be used to examine the relationship between the parameters of the observed heterogeneity distribution and pathogen ESS virulence, such as the median trait or variation in that trait. For example, a change in ESS virulence attributable to a change in the median trait alone (a pure location shift) can be examined by comparing two homogeneous populations with a different trait value, while a change in ESS virulence attributable to just a change in variance can be quantified by comparing a homogeneous population with a given trait value to a heterogeneous population with the same median. The latter scenario can be used to examine what we term *median-exploitation*, which quantifies the amount of over- or under-exploitation experienced by a *typical host* (i.e. a host with a trait value at the median of the heterogeneity distribution) that is directly attributable to host heterogeneity. A direct comparison of ESS virulence in a heterogeneous population to a reference population with trait value equal to zero involves changes in both median and variance, and therefore the predicted change in ESS virulence cannot be attributed to host heterogeneity. Nonetheless, this comparison is useful for predicting ESS virulence in rabbit populations from empirical data, without an explicit decomposition of the relative effect of a change in median and change in variance.

From a pathogen-centric view, our model can be used to determine how host heterogeneity affects a pathogen's ability to spread in the host population. This can be quantified as the fold (multiplicative) change in pathogen R_0 that results from exploitation of the heterogeneous population, a quantity we refer to as pathogen *efficiency*. Efficiency quantifies the cost to the pathogen for exploiting a heterogeneous host population. In our case

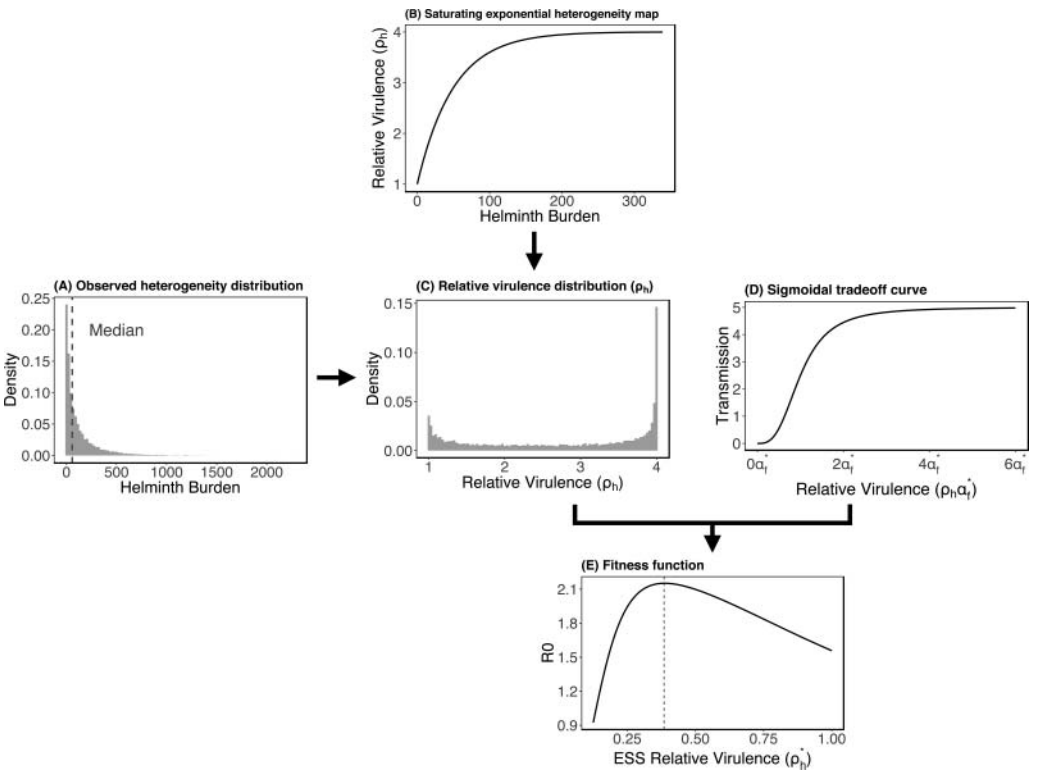

Fig. 2. Model schematic. Panel A shows the population distribution of helminth burden, our measure of host heterogeneity. Panel B shows a saturating-exponential heterogeneity map. Panel C shows the population distribution of relative virulence (ρ_h: the fold-increase in virulence relative to a helminth-free rabbit) that results from translating the distribution in A through the heterogeneity map in B. Together the ρ_h distribution (panel C) and the trade-off curve (panel D: a sigmoidal curve in this example) determine the R_0 value at different levels of pathogen relative virulence (panel E). The horizontal axis in panel E shows the virulence of a given pathogen strain in the heterogeneous population relative to ESS virulence in the homogeneous population, with the dotted line showing ESS relative virulence in the heterogeneous population (ρ_h^*).

study, efficiency is defined as the R_0 of the ESS strain in the heterogeneous population divided by the R_0 of the ESS strain in a homogeneous host population. Because we assume the pathogen always reaches its ESS virulence, R_0 and thus efficiency is unaffected by the median trait value, and therefore it is just a function of host heterogeneity.

Patterns in ESS virulence, over- or under-exploitation of a typical host, and efficiency can be explored generally using the *shiny* R applications (Chang *et al.*, 2017) in the Appendix (evolutionary-ecology.com/data/3118Appendix.pdf). These applications allow users to select parameter values and visualize model outcomes in a layout similar to Fig. 2. Users can explore these applications on their own or paired with the general model exploration presented in the Appendix. The material there describes in detail the qualitative patterns we found most interesting while exploring the model, including details about qualitative differences in results between the power-law and sigmoidal trade-off curve, and between the

saturating exponential and piecewise heterogeneity map. Parameter values for the results presented in the Appendix are available in Table S1.

In the main text we instead focus more narrowly on applying our model to the *O. cuniculus*–myxoma virus system.

The *O. cuniculus*–myxoma virus case study

The initial release of MYXV in Australia and Europe in the 1950s caused unprecedented rabbit mortality: case mortality for the Standard Laboratory Strain (SLS) was ~99%, with an infected rabbit's average lifespan under 13 days (Fenner, 1953). However, within three years of its release, MYXV evolved to intermediate virulence on both continents (Kerr *et al.*, 2012). It is well established that attenuation of the myxoma virus occurred due to a combination of increased resistance in rabbits and higher fitness of lower virulence myxoma strains (Kerr *et al.*, 2015). Yet, many fundamental aspects in the evolution of MYXV virulence remain unresolved, including the impact of heterogeneity in host susceptibility to MYXV infection. In natural settings, rabbits are commonly infected with a community of gastrointestinal helminths that interact directly or indirectly within the host (Lello *et al.*, 2004; Cattadori *et al.*, 2008, 2014; Murphy *et al.*, 2013) and can affect the virulence and transmission of MYXV (Kerr *et al.*, 2004).

Detailed information on the interaction of MYXV and helminths, such as the effects of helminth burden on MYXV infection severity and colonization within the host, is currently unavailable. However, it is known that helminths, including the common gastrointestinal species found in rabbits, cause an upregulation of the type-2 immune response (Cattadori *et al.*, 2007, 2016; Murphy *et al.*, 2011, 2013; Thakar *et al.*, 2012), which has antagonistic components to the type-1 reaction developed against viruses and bacteria, including MYXV (Nash *et al.*, 1999; Kerr *et al.*, 2004; Cattadori *et al.*, 2007). These contrasting immune responses impair the host's ability to successfully control both infections (Kerr *et al.*, 2004; Cattadori *et al.*, 2007). Therefore, we would expect infection by helminths to increase the mortality of rabbits infected with MYXV. Under this scenario, gastrointestinal helminths might have contributed to the rapid attenuation of MXYV virulence by causing the accelerated mortality of co-infected rabbits and facilitating the selection for less virulent MYXV strains that allow longer host survival. We assess this strict type-1/type-2 immune response trade-off (Cattadori *et al.*, 2007), dependent on the total helminth burden (but irrespective of helminth species) using the saturating heterogeneity map.

The alternative scenario is where low (but non-zero) helminth burdens may increase the ability of rabbits to cope with MYXV infection. Rabbits that have co-evolved with helminths for centuries (Audebert and Durette-Desset, 2007), and with MYXV for decades (Fenner and Fantini, 1999), have developed some resistance to the virus, and can manage co-infections with both when helminth intensity is not too high or MYXV is not too virulent (Cattadori *et al.*, 2007, 2008; Kerr *et al.*, 2017). Indeed, observations of wild rabbits from recent populations show that rabbits can cope with both infections (Cattadori *et al.*, 2007). These observations suggest that low to intermediate helminth burdens may train (prime) the host immune response to deal with both infections to the point of being beneficial to the host in coping with the MYXV infection (Kemp and Björkstén, 2003; Okada *et al.*, 2010; Graham *et al.*, 2011). At intermediate to high helminth burdens, the effect of the type-1/type-2 immune response trade-off is assumed to outweigh the benefit of a primed immune reaction, leading to an increase in MYXV virulence in rabbits with intermediate to high helminth burdens. We model this scenario

using the non-monotonic piecewise heterogeneity map, which allows for rabbits with low to intermediate helminth burdens to have *reduced* MYXV virulence relative to helminth-free rabbits but retains the saturating exponential relationship between helminth burden and MYXV virulence at high helminth burdens, which captures the type-1 and type-2 response trade-off. Under this general scenario, a helminth burden distribution with a low median would select for higher MYXV virulence to allow the virus to escape the immune response and achieve maximum transmission.

To explore the effects of these biological scenarios, we first examine qualitative patterns in MYXV ESS virulence under both scenarios across a broad parameter set. We focus on MYXV ESS virulence as a function of the two heterogeneity maps and helminth burden distributions and examine an isolated change in both median and variance in helminth burden. Here we consider only a sigmoidal trade-off relationship between virulence and transmission of MYXV fit to data on the relationship between virulence, transmission, and recovery rate; more cases are considered in the Appendix. The quantitative relationship is taken from Fenner and Marshall (1957; presented in Dwyer *et al.*, 1990) and estimated using non-linear least squares [with the nlxb function in the package nlmrt (Nash, 2016)].

Second, we examine the possibility that a non-monotonic relationship between helminth burden and MYXV virulence could explain current-day local MYXV strain diversity and historical differences in virulence between the UK and Australia (Appendix Fig. S2). Phylogenetic studies from recent rabbit populations of Australia and the UK have found that highly virulent strains are currently co-circulating with attenuated strains (Kerr *et al.*, 2012, 2015, 2017). There is also evidence that the average virulence of strains has been historically higher in the UK than in Australia (Fig. S2). An exploratory statistical analysis over years 4–30 after release, ignoring the first 4 years following release (a poorly sampled 4-year transient period), shows that the average case mortality of UK MYXV strains has been higher than Australian strains on average between 1955 and 1985 (Australia 95% CI: 0.73–0.77, UK 95% CI: 0.82–0.89; $P < 0.05$; generalized linear model with binomial error distribution and numbers of strains sampled as weights). Differences in the gastrointestinal helminth burden may help to explain these results. We examine what combinations of parameters for the location of the piecewise minimum (h) and the maximum reduction of MYXV virulence (k) must be assumed for heterogeneity in gastrointestinal helminth burden to generate higher ESS virulence. Here, h determines the helminth burden at which relative MYXV virulence is the lowest, where this minimum virulence is given by $k(0 < k < 1)$. Beyond this helminth burden, relative MYXV virulence follows the saturating exponential curve. We use helminth burden estimates from three sites in Australia (Dunsmore, 1966) and one site in Scotland (Cattadori *et al.*, 2007). We note that the historic trend of higher MYXV virulence in the UK (Fig. S2) is not directly associated with the Scotland site, and that the helminth burdens measured at the Scotland site may not be representative of helminth burdens across the UK.

Computational methods

We write ESS virulence in a homogeneous reference population as α_f^* and in a heterogeneous population as α_h^*. We define α_h^* as $\alpha_f^* \rho_h^*$, where we call ρ_h^* the ESS relative virulence. In the reference population, optimal virulence α_f^* is calculated by setting the derivative of the R_0 expression to 0 and solving for α; we denote the corresponding R_0 as R_{0f}. To determine α_h^*, we first translate the distribution of helminth burden into a distribution of pathogen

virulence among hosts using the given heterogeneity map. With our assumption of a perfectly implastic pathogen, an intrinsic pathogen exploitation strategy results in a different realized virulence in each individual in the heterogeneous population. We refer to pathogen virulence in a given individual as α_h, and to the distribution of α_h values as the *realized virulence distribution*. We define α_h as $\alpha_f^* \rho_h$, where ρ_h is relative virulence in a particular host. We refer to the distribution of ρ_h values as the *relative virulence distribution*. A given pathogen exploitation strategy will result in a population-level R_0 that depends on the distribution of $\alpha_h(\rho_h)$. A pathogen with virulence α_f^* in a reference host has an R_0 in a heterogeneous population given by the following integral:

$$R_0 = \int_0^\infty \frac{\beta(\alpha_f^* \rho_h)}{\mu + \alpha_f^* \rho_h} P(\rho_h)\, \mathrm{d}\rho_h,$$

where $P(\rho_h)$ is the distribution of relative virulence in the heterogeneous population, and $\beta(\alpha_f^* \rho_h)$ is given by the trade-off curve.

We solve this integral numerically in R and find the level of pathogen virulence that optimizes R_0 in the heterogeneous population ($\alpha_h^* = \alpha_f^* \rho_h^*$) using the optimize function. Like previous models, this method for determining pathogen ESS virulence results in a pathogen that adopts a life-history strategy tailored to hosts that contribute the most to the pathogen's reproductive potential – called 'high quality' hosts by Gandon (2004) and 'prime hosts' by Pugliese (2011) – weighted by host abundance. We refer to the R_0 of the pathogen in the heterogeneous population with virulence α_h^* as R_{0h}. In this notation, relative virulence is expressed as $\rho_h^* = \alpha_h^*/\alpha_f^*$, median-exploitation is defined by ρ_h^* in the presence of changing heterogeneity without a change in the median, and efficiency is expressed as R_{0h}/R_{0f}. Table S1 shows parameter values used in the general model exploration presented in the Appendix as a companion to the *shiny* R application, as well as parameters used in our examination of the *O. cuniculus*–myxoma virus case study.

RESULTS

General model exploration: qualitative patterns

In our general model exploration, we focus on the effects of the shape of the trade-off curve, heterogeneity map, and host trait distribution on qualitative patterns in ESS virulence and efficiency, as well as robust patterns in the sensitivity of these model outcomes to changes in the parameter values. In brief, we find that an increase in host heterogeneity to infection (variance) always decreases the ability for a pathogen to spread in the host population, but that the amount of pathogen efficiency loss depends strongly on the shape of both the trade-off curve and the heterogeneity map. An increase in the slope of either trade-off curve decreases parasite R_0, although the change in R_0 is larger and more sensitive to changes in the slope of the sigmoidal trade-off curve. Both ESS virulence and the amount of over- or under-exploitation of a typical host also depend on the shape of the trade-off curve. For example, for the saturating exponential heterogeneity map and for a given host heterogeneity distribution, increasing the slope/curvature (γ) of the power-law trade-off curve decreases ESS relative virulence, while an increase in the slope of the sigmoidal trade-off curve at its inflection point increases ESS relative virulence and over-exploitation of a typical host (Fig. S3).

These patterns as well as the effects of the shape of the heterogeneity map and observable heterogeneity distribution are presented in the text of the Appendix, and can be explored visually using the online *shiny* R applications presented there.

The *O. cuniculus*–myxoma virus case study

Might co-infection with gastrointestinal helminths have contributed to the observed post-introduction attenuation of MYXV virulence?

Any saturating heterogeneity map capturing the type-1/type-2 immune response trade-off always predicts lower ESS virulence (i.e. MYXV attenuation) in a population of rabbits with a non-zero helminth burden, regardless of the shape of the helminth burden distribution. However, the relative importance of changes both in the median and variance of helminth burden depends on the shape of the heterogeneity distribution. For example, with the saturating heterogeneity map shown in Fig. 2 adding a helminth burden homogeneous to all rabbits of 56.9 parasites (this value is the median of the Gamma distribution pictured in Fig. 2) leads to a 3.05-fold decrease in MYXV virulence. Adding a heterogeneous worm burden following the Gamma distribution pictured reduces ESS virulence by a factor of 2.50. Thus, the effect of heterogeneity is to moderately *increase* MYXV virulence. Similar but variable increases in ESS virulence attributable to heterogeneity are seen with different Gamma distributions and can be examined using the online *shiny* R applications. Illustrations of the relative effects of changing the median and the variance are available in Figs. S3 and S4. The Gamma distribution of helminth burden does not allow for a median equal to zero and positive variance, so we cannot compare a homogeneous population with a median equal to 0 and a heterogeneous population with a median equal to 0.

In contrast, a non-monotonic heterogeneity map that models a decrease in MYXV virulence at low to intermediate helminth burden greatly reduces the parameter space leading to MYXV attenuation. Instead, it more commonly selects for an *increase* in MYXV ESS virulence relative to a homogeneous rabbit population without helminth parasites. For example, with the helminth burden distribution for Urana, NSW, and the parameters for the saturating exponential proportion of the piecewise heterogeneity map used in Fig. 2 ($z = 3$, $r = 0.020$), the addition of the quadratic portion of the piecewise heterogeneity map leads to an increase in MYXV ESS virulence at most parameter values for k and h (Fig. 3). Alternative values for z and r can be explored using the *shiny* R application in the Appendix.

When partitioning the relative impact of a change in the median and a change in the variance of the helminth burden distribution on ESS virulence using a piecewise heterogeneity map, we find that a change in variance has the largest effect when the median of the heterogeneous host population is near the minimum of the quadratic portion of the piecewise heterogeneity map. In this case a homogeneous population with helminth burden near the quadratic vertex experiences a MYXV strain with higher virulence than a population with zero helminth burden. Here, an increase in the variance of the helminth burden without a change in the median increases the proportion of the rabbit population with high helminth burdens who select for a decrease in MYXV virulence; a large increase in the variance can switch ESS virulence from >1 (higher virulence) to <1 (lower virulence, i.e. attenuation). For example, with a piecewise heterogeneity map with parameters $z = 3$, $r = 0.02$, $h = 21$, $k = 0.6$, a homogeneous rabbit population with a helminth burden of 20.33

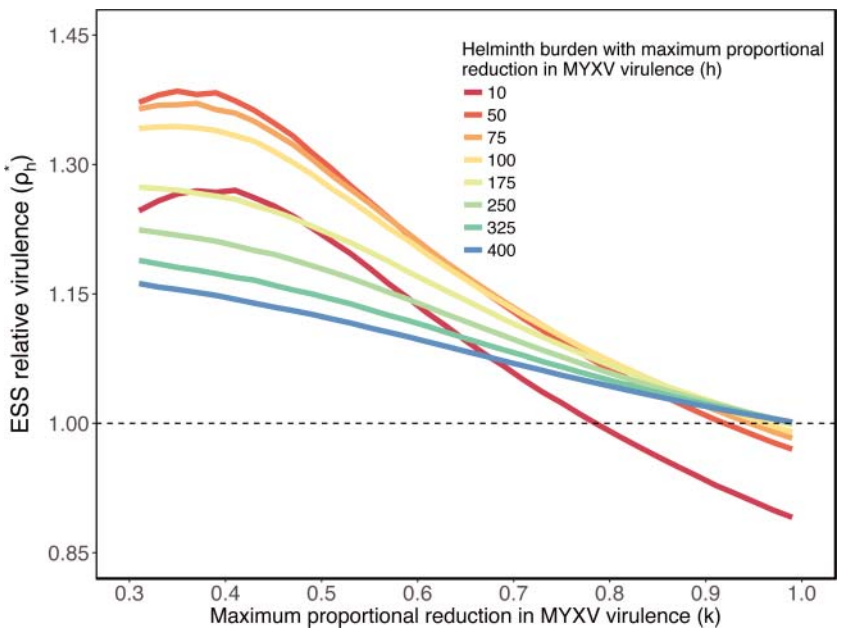

Fig. 3. ESS relative virulence, ρ_h^*, given by the ratio of ESS virulence in the heterogeneous rabbit population (α_h^*) to ESS virulence in a homogeneous helminth-free rabbit population (α_f^*). Results shown here use the helminth burden distribution fit to Urana, NSW and a piecewise heterogeneity map (saturating exponential portion: $z = 3$, $r = 0.02$ as pictured in Fig. 2; quadratic portion: $k = 0.31$–0.99, $h = 10$–400). The x-axis shows a range for k, which is the proportional reduction in MYXV virulence at the quadratic vertex. Colours show k, which is the helminth burden at the vertex of the quadratic portion of the piecewise heterogeneity map.

parasites results in a 2.5× *higher* ESS virulence of MYXV than in a homogeneous rabbit population without helminths. However, in a rabbit population with a helminth burden following a Gamma distribution with a coefficient of variation of 1.83 and a median of 20.33 parasites (scale = 278, shape = 0.3), MYXV ESS virulence is 1.11-fold *lower* than in a homogeneous population with no helminths. This pattern is presented visually in Fig. S4.

Under what conditions can co-infection with helminths facilitate higher ESS MYXV virulence?

Given that a non-monotonic relationship between helminth burden and MYXV virulence can increase MYXV ESS virulence in a rabbit population with helminths, the difference in virulence in MYXV strains circulating in Scotland and Australia could be explained by the differences in helminth burdens in these two countries. In this section, we examine the parameters for the non-monotonic heterogeneity map that could support differences in ESS virulence of the magnitude seen in Australia and Scotland.

Only an intermediate reduction in virulence (e.g. magnitude of MYXV virulence reduction $k = 0.4$) at a high helminth burden (e.g. helminth burden at virulence, minimum $h = 300$) selects for higher ESS virulence in Scotland than in Snowy Plains, NSW (with a peak of ~1.10× higher virulence) (Fig. 4). At all other parameter combinations, virulence is

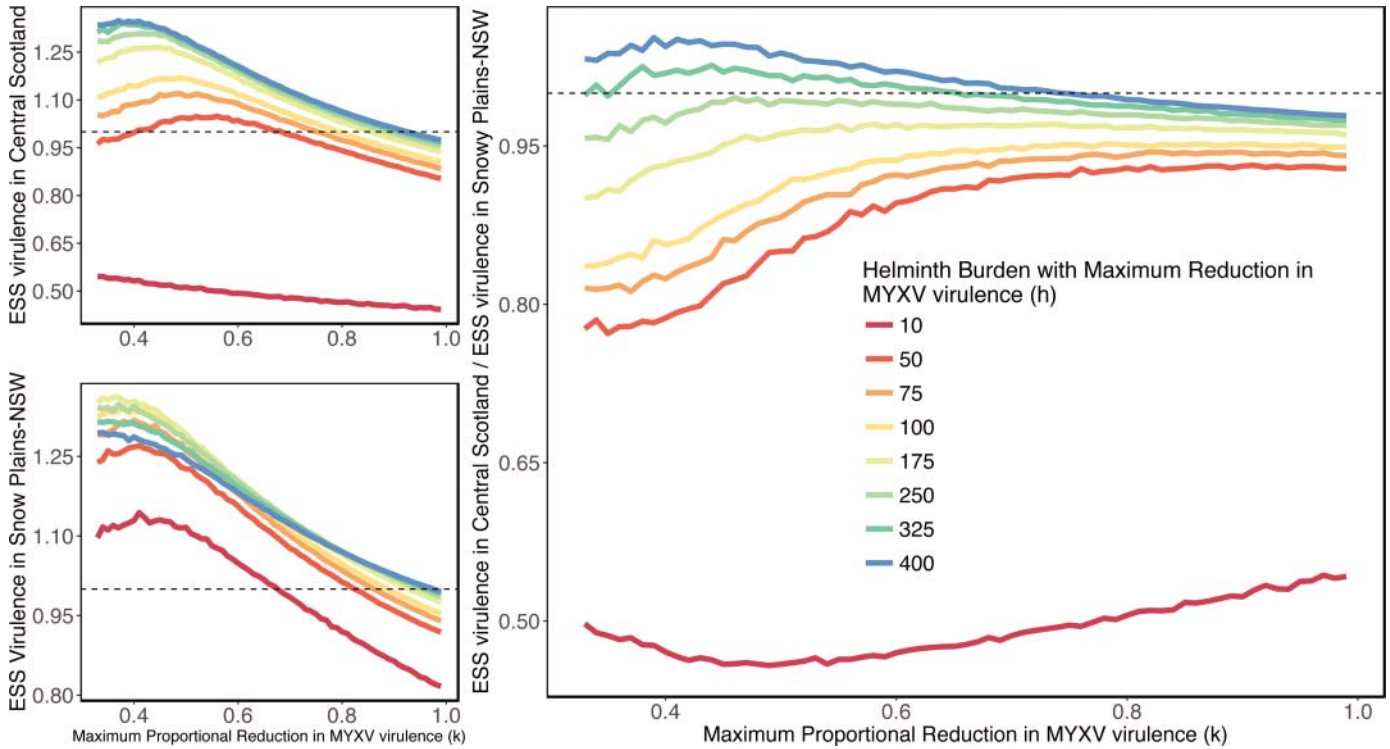

Fig. 4. Panels A and B show the fold change in MYXV virulence in Central Scotland and Snowy Plains, NSW relative to MYXV virulence in a homogeneous helminth-free rabbit population. In panels A and B, regions above and below the dashed reference line show parameter combinations that lead to a higher or lower ESS MYXV virulence in the wild rabbit populations, respectively. Panel C shows the ratio of ESS virulence in Scotland relative to ESS virulence in Snowy Plains, NSW. Here, regions above and below the dashed reference line show parameter combinations that lead to higher or lower virulence in Scotland, respectively. Parameter values for the piecewise heterogeneity map are the same as those used in Fig. 3 ($z = 3$, $r = 0.02$, $k = 0.31$–0.99, $h = 10$–400).

higher in Snowy Plains, NSW. A rapid change in the ratio of ESS virulence between Scotland and Australia occurs between a piecewise minimum (h) of 10 and 50, respectively. This range of precipitous change occurs because of a high variance in the relative virulence distribution in the Snowy Plains, NSW population. Similar qualitative results occur using different parameter values for the piecewise heterogeneity map (Fig. S5).

Comparing the rabbit population from Scotland and rabbit populations in other locations in Australia (either Urana, NSW or Mitchell, Queensland), the parameter space for h and k that leads to higher ESS virulence in Scotland is slightly larger than for Snowy Plains, NSW (Fig. S6). The larger difference between the Urana, NSW or Mitchell, Queensland populations and Scotland (Snowy Plains has the most similar helminth burden to Scotland of the three Australian populations), supports a larger range of parameters for the heterogeneity map that selects for higher ESS virulence in Scotland (Fig. S6). As the difference in median helminth burden between the two countries increases, a smaller proportion of the two helminth burden distributions overlap the same region of the heterogeneity map, which leads to divergent responses in the populations from the two countries. This allows for more heterogeneity maps that have a quadratic portion spanning the range of helminth burdens found in the Australian but not in the Scotland population, which selects for higher virulence in Scotland.

DISCUSSION

We have presented a general model on the evolution of virulence in heterogeneous host populations that retains the fundamental structure and emergent properties of previous models (e.g. Gandon *et al.*, 2001; Pugliese, 2011) but has been structured to be more easily parameterized with empirical data (Alizon and Michalakis, 2015; Cressler *et al.*, 2016). Specifically, experimental or observational data are needed to parameterize the distribution of the focal host trait that influences infection (host heterogeneity), the heterogeneity map between pathogen virulence and the host trait, and the transmission–virulence trade-off curve. In the *O. cuniculus*–MYXV system, the distributions of gastrointestinal helminths in wild rabbit populations were calculated from sampling wild animals (Dunsmore, 1966; Cattadori *et al.*, 2007). Experiments to parameterize the heterogeneity map remain incomplete. In the end, two separate experiments will be needed in the *O. cuniculus*–MYXV system to parameterize the heterogeneity distribution and heterogeneity map. However, with an *a priori* goal of model parameterization, the distribution of host heterogeneity could be collected in conjunction with an experiment designed to parameterize the heterogeneity map. The sigmoidal trade-off curve in the *O. cuniculus*–MYXV system was parameterized in two steps using laboratory experiments performed previously. First, viral loads and the lifespan of infected rabbits were determined by examining the survival of laboratory animals infected with a diversity of MYXV strains (Fenner and Marshall, 1957). Second, the relationship between viral load and vector transmission was determined by allowing mosquitoes to feed on a rabbit at the site of the primary skin infection and calculating the probability of MYXV transmission to an uninfected rabbit (Fenner *et al.*, 1956). In the case of a directly transmitted disease, both transmission and host mortality could be measured in the same experiment (De Roode *et al.*, 2008).

Since no host–pathogen system currently provides sufficient data to parameterize the entire model, including the *O. cuniculus*–MYXV system, we first explored the qualitative effects of different shapes of the heterogeneity map and the virulence–transmission trade-off curve on ESS virulence, median-exploitation, and pathogen efficiency. Second, we

used our model to examine the effects of host heterogeneity to helminth infection on viral evolution in the *O. cuniculus*–MYXV system. We examined whether helminths could have been important drivers of the initial rapid attenuation of MYXV virulence in Australia and Europe (Kerr *et al.*, 2004; Cattadori *et al.*, 2007), and/or of the prolonged difference in MYXV virulence between these two continents (Fig. S2) (Kerr *et al.*, 2012, 2015, 2017). Because the exact effects of helminth co-infection on MYXV virulence are unknown, we investigated two possibilities. First, based on the general concept of the dichotomy between the type-1 and type-2 immune response trade-off to micro- and macro-parasites, we examined a monotonically increasing but saturating relationship between helminth burden and MYXV virulence. Second, motivated by observations of rabbits in the field (Cattadori *et al.*, 2007), we examined a non-monotonic relationship between helminth burden and MYXV virulence that modelled lower realized MYXV virulence in rabbits co-infected with low to intermediate helminth burdens, where the immune system has already been primed.

Model construction and assumptions

The heterogeneity map (which describes the relationship between a measurable host trait and pathogen virulence) and the distribution of host heterogeneity for a focal trait are key drivers of changes in relative virulence, median-exploitation, and efficiency (i.e. ability to transmit). These two model components affect results directly through their joint control of the relative virulence distribution (ρ_h, Fig. 2), and indirectly by regulating the impact of the functional form (power-law or sigmoidal) and slope of the trade-off curve. The shape of the trade-off curve has the largest impact on relative virulence, median-host exploitation, and efficiency (Fig. S3) when variance in the relative virulence distribution is maximized, which occurs when the relative virulence distribution is bimodal (Fig. 2). This can occur, for example, when the heterogeneity map approaches its asymptote near the mean of the host heterogeneity distribution (Fig. 2). With a bimodal relative virulence distribution, any change in either the measured host heterogeneity distribution or the heterogeneity map causes a shift in which mode of the bimodal relative virulence distribution has higher density. For example, in the extreme of a relative virulence distribution composed of half low-virulence and half high-virulence extremes (e.g. a population with 50% helminth-free rabbits and 50% rabbits with heavy helminth burden), a change to a distribution of either 51%:49% or 49%:51% causes a shift in the preferred host type and a large difference in model outcomes. That is, a bimodal relative virulence distribution will result in an implastic pathogen specializing on the marginally more abundant host type, leading to large efficiency losses because of extreme over- or under-exploitation of the other host types. Although we do not explicitly model the case of two discrete host types, a bimodal relative virulence distribution that approximates a host population with two host types has been previously examined (e.g. Gandon *et al.*, 2001; Gandon, 2004). Additionally, while our model is limited to a single monomorphic strain, a bimodal relative virulence distribution favours the evolution of a specialist pathogen on a subset of the host population (Regoes *et al.*, 2000). In the opposite extreme of a uniform relative virulence distribution, the ESS pathogen strategy is to specialize on hosts with a mean trait value. In this scenario, changes in the distribution of helminths or the shape of the trade-off curve have little effect on model outcomes.

Because of the dependence of the relative virulence distribution on the shape of the heterogeneity map, our main conclusions depend strongly on our assumption of a saturating heterogeneity map (which holds for both the saturating exponential and the piecewise

maps used here). Specifically, a saturating function increases the range of population heterogeneity distributions that create a bimodal distribution of relative virulence. This not only increases the sensitivity of model outcomes to changes in population heterogeneity, but also increases the sensitivity of results to the shape of the trade-off curve. In our case study, the relationship between helminth burden and MYXV virulence is likely not perfectly saturating (i.e. the virus–helminth interaction does not produce a true ceiling on MYXV virulence), though a saturating function is a plausible first approximation of the effects of co-infection and other sources of host heterogeneity. Alternative scenarios can be smoothly incorporated into the model framework. For example, it is possible to specify a power-law curve that decelerates (negative second derivative) but increases indefinitely (no asymptote).

At one extreme, if the full range of the heterogeneity distribution spans only the approximately linear portion of the heterogeneity map, the relative virulence distribution will resemble a scaled heterogeneity distribution, reducing the importance of the shape of the trade-off curve. At the other extreme, when the heterogeneity distribution spans the full range of the heterogeneity map such that an appreciable portion of its density (e.g. 30%) is near the asymptote of the heterogeneity map, variance in the relative virulence distribution will be maximized (see Fig. 2), and the shape of the trade-off curve increases in importance. Together, these results imply that while a decelerating curve has the potential to create a bimodal relative virulence distribution, it will greatly depend on the slope of the curve within the range of the heterogeneity distribution.

Previous studies have gathered data on individual heterogeneity in traits similar to those needed to parameterize the heterogeneity map, including host size (Cable and Van Oosterhout, 2007), nutritional status (Bedhomme *et al.*, 2004), and maternal environment (Stjernman and Little, 2011; Garbutt *et al.*, 2014). However, the data from most of these studies are insufficient for our model because they are based on experiments that used two discrete host groups (ANOVA design) instead of a treatment range [regression design (Inouye, 2001; Cottingham *et al.*, 2005)]. Despite noisy data, there is some preliminary empirical evidence for a saturating heterogeneity map (Cable and Van Oosterhout, 2007), but also for a linear relationship between food availability and virulence, at least within the range examined (Bedhomme *et al.*, 2004). Regardless, more empirical work that follows a regression design is needed for parameterization of the heterogeneity map. One promising case is the *Daphnia magna–Pasteuria ramosa* system, in which virulence differs between low and high rearing temperature (Garbutt *et al.*, 2014) and where a trade-off curve has been quantified (Jensen *et al.*, 2006).

In an effort to keep our model simple enough to be readily parameterized with experimental data, and given the ecological specifics of the *O. cuniculus*–MYXV system, we ignore the evolution of pathogen specialists (Regoes *et al.*, 2000), as well as complexities such as pathogen plasticity (Gandon, 2004; Fleming-Davies *et al.*, 2015), host–pathogen co-evolution (Pugliese, 2011), or the emergence of individual heterogeneities during the course of the infection (Osnas and Dobson, 2012). Our assumption that the focal pathogen is perfectly implastic causes heterogeneity to maximally increase over- and under-exploitation in a subset of hosts; a perfectly implastic pathogen will experience the highest possible efficiency loss in a heterogeneous population. Alternatively, a completely plastic pathogen will change its virulence to match any value of the relative virulence distribution, preventing efficiency loss in a heterogeneous population. While we did not examine pathogen plasticity, it has recently been shown that pathogen plasticity, in the presence of host heterogeneity, can lead to the co-existence of high and low virulence strains (Fleming-Davies *et al.*, 2015). The lack of plasticity shown by MYXV makes this an implausible explanation for the variation in virulence of circulating

MYXV strains, but may be important to consider in other systems. The model framework can incorporate other assumptions for pathogen plasticity by allowing the pathogen's intrinsic exploitation strategy to vary depending on the host type. For example, a single additional scaling parameter could be added to the model that pulls a host's effective virulence (α_h) towards the intrinsic exploitation strategy of the pathogen, which would reduce the impact of heterogeneity.

In the *O. cuniculus*–MYXV system, the trade-off between transmission and virulence is the dominant constraint affecting pathogen evolution, though the original formulation of the trade-off using this system actually used the trade-off between transmission and recovery (Anderson and May, 1982). This trade-off may be more important in other systems than the trade-off we model here (Alizon, 2008; Cressler *et al.*, 2016). While the fundamental structure of our model does not need to change to incorporate a transmission–recovery trade-off, adjustments to each model component are needed. The equation for R_0 would need to be rewritten to focus on recovery, and both the heterogeneity distribution and the heterogeneity map would need to focus on traits associated with recovery rather than virulence. The computational methods for finding the optimum pathogen exploitation strategy would be applied as described here.

Finally, we ignore the possibility that changes in MYXV virulence, a vector-transmitted disease, affect both transmission from an infected rabbit to a susceptible mosquito/flea and transmission from an infected mosquito/flea to a susceptible rabbit. Given that mosquitoes or fleas act as mechanical vectors for MYXV, changes in virulence are not expected to affect the vector in this system. However, in other systems pathogen virulence in the host and pathogen virulence in the vector could be coupled to some degree. Ignoring this coupling could potentially lead to an incorrect estimate of the optimal pathogen strategy.

The *O. cuniculus*–myxoma virus case study

This study was in part motivated by our interest in the role of helminth infections in the attenuation of MYXV following its initial release in Australia and Europe in the 1950s. It is well understood that MYXV evolved towards a more attenuated virulence on both continents (Kerr *et al.*, 2012) because of selection against both highly virulent strains that killed rabbits too quickly and low virulence strains that produced too little virus for transmission by vectors (Fenner and Marshall, 1957; Dwyer *et al.*, 1990). The parallel increased resistance of rabbits to MYXV was an additional cause of reduced virus transmission (Kerr *et al.*, 2015). We argue that helminths might have contributed to the initial attenuation of MYXV by having susceptible rabbits with an already impaired immune response to the virus experience faster mortality. To capture this immune alteration, specifically the type-1/type-2 antagonistic immune reaction between MYXV and helminths (Nash *et al.*, 1999; Cattadori *et al.*, 2007), we used a saturating function to describe the heterogeneity map. A saturating heterogeneity map always predicts lower ESS virulence in the presence of helminths regardless of the shape of the heterogeneity distribution and trade-off curve. Critically, partitioning the effects of a change in the median helminth burden from a change in the variance of helminth burden, under a strict type-1/type-2 immune response trade-off, we found that an increase in the heterogeneity of helminth burden without a change in the median tends to decrease ESS virulence. Thus, any effect of MYXV attenuation attributable to helminths under the scenario of a strict type-1/type-2 trade-off is caused by an increase in the overall helminth burden in the population (measured here using the median).

Under the assumption of reduced MYXV virulence in rabbits infected with a range of helminth burdens, which we modelled using a piecewise heterogeneity mapping function, ESS virulence can be higher or lower than in a homogeneous population (Figs. 3 and S4) depending on the shape of the heterogeneity map. Here, an increase in variation without a change in the median can decrease ESS virulence substantially, potentially reversing the overall change in virulence from an increase (comparing between a homogeneous population with no helminths and a homogeneous population with helminths) to a decrease (comparing between a homogeneous population with helminths to a heterogeneous population with median helminth burden equal to the helminth burden in the homogeneous population).

Together, these results suggest that little can be said definitively. There is an urgent need for experiments that quantify the shape of the heterogeneity mapping function in order to provide a conclusive answer to the possibilities presented here. For example, laboratory experiments of MYXV–helminth co-infections can quantify the MYXV replication rate and virulence in rabbits infected with different helminth burdens. With the ability to parameterize the heterogeneity map, this simple heterogeneity model could be combined with a more detailed epidemiological model (e.g. Dwyer *et al.*, 1990) for MYXV transmission to capture more realistically the trajectory and causes of the attenuation of MYXV following the initial introduction as well as the current patterns of circulating strain diversity. Tentatively, we argue that the most realistic scenario is that co-infections, in concert with an increase in rabbit resistance, would have contributed to the decrease of MYXV virulence in wild rabbits following the release of the virus, but further work is needed to support this conclusion.

We also examined whether lower MYXV virulence in rabbits with intermediate helminth burden relative to helminth-free rabbits could explain the approximately 1.2 times higher virulence of circulating MYXV strains in Scotland than in Australia in the decades following the release of the virus in both countries (Fig. S2). Using a piecewise function for the heterogeneity mapping function, we show that there is a relatively restricted and moderately extreme parameter set that selects for higher virulence in a population of rabbits with higher mean helminth burden. For example, minimum MYXV virulence has to occur above 300 helminths, which is much higher than the median of 138 helminths estimated from the Gamma distribution fit to the helminth burdens measured in Scotland (Cattadori *et al.*, 2007). These 300 helminths must also be accompanied by a reduction in MYXV virulence of ~50% (Fig. 4). Given the prevalence of both MYXV and helminths in Scotland and the much larger effect MYXV has on rabbit mortality relative to helminths, including the evolution of increased host resistance to MYXV on a time frame of decades, it is unlikely that a reduction of 50% in virulence would be maintained in the host population without evolution towards increased susceptibility to helminths. Smaller reductions in MYXV virulence (k) can also lead to greater ESS virulence in Scotland, but only at even larger helminth burdens (Fig. 4). For example, with $h = 400$, virulence in Scotland is only a maximum of ~1.1 times that in Snowy Plains, NSW (Fig. 4), providing further support against helminths as a causal factor in selecting for higher MYXV virulence.

A comparison between Scotland and Urana, NSW (a population with lower helminth burden than the Snowy Plains, NSW population), a larger parameter space supports higher virulence in the Scotland population (Fig. S6). If the Urana, NSW population was isolated from the rest of Australia, a mediated helminth-controlled reduction in relative MYXV virulence could be a plausible explanation for differences in MYXV virulence between the

populations. However, with the mixing of rabbit populations and the equally regional spread of MYXV by mosquitoes and fleas, it is unlikely that the shape of the heterogeneity map varies by population. Therefore, while a given heterogeneity map could plausibly explain why virulence is higher in Scotland than a single region of Australia, this explanation is brittle in the face of a well-mixed host population on the continental scale. Yet, this does not rule out the possibility that some of the spatial diversity observed in MYXV virulence (Kerr *et al.*, 2010, 2015) is driven by interpopulation variation in helminth burden. Little is known about the rate of strain emergence or extinction, or the scale at which strains circulate, though recent work indicates new strains are gained and lost each year (Kerr *et al.*, 2017). Our simulations presented in Figs. 3 and 4 suggest that differences in the distribution of helminth burden can select for large differences in ESS virulence and could in part be driving the patterns of the high spatial diversity in MYXV virulence (Kerr *et al.*, 2010, 2015).

To improve our model, the within-host dynamics of MYXV in rabbits free of helminths can provide a baseline of the fundamental processes of virus evolution. The empirical trade-off curve used here, derived from laboratory data (Fenner and Marshall, 1957), does not capture the important biological MYXV–rabbit interactions that directly affect virulence. Indeed, the use of viral titre measured at the site of the mosquito skin lesion [fibroma (Dwyer *et al.*, 1990)] leads to a range of values for peak titre or area under the curve [two standard methods of measuring transmission potential (Handel and Rohani, 2015)], resulting in two possible values of both case mortality and transmission (i.e. two values from a non 1 : 1 function). Ultimately, more details on the dynamics of infection within rabbits and the role of secondary skin lesion in MYXV transmission are needed if we are to develop more realistic evolutionary models of infection.

CONCLUSION

The mapping function of host heterogeneity to pathogen virulence has a marked influence on specific outcomes of interest. In the absence of empirical data, we caution that assumptions about its characteristics, and to a lesser extent, the shape of the transmission–virulence trade-off curve, can have large effects on model outcomes. While our simulations, like others, are largely driven by model assumptions (Pugliese, 2011), the ease of substitution of functional forms in our model will help bridge the gap between theory and data in this field. As Alizon and Michalakis (2015) and Cressler *et al.* (2016) indicate, the field drastically needs more studies that incorporate knowledge of the full life cycle of both host and pathogen. Infection with a second pathogen species is likely to be a large part of the host's life cycle as well as modulate the life history and traits of the focal pathogen (Graham *et al.*, 2007; Fenton, 2008; Thakar *et al.*, 2012; Cattadori *et al.*, 2014) and ultimately pathogen evolution.

ACKNOWLEDGEMENTS

We thank the Dushoff lab and Jo Werba for helpful comments on the first draft of the manuscript. This work was funded by NSERC Discovery Grant 386590-2010.

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