

**IDEA AND  
PERSPECTIVE**

# Applying population-genetic models in theoretical evolutionary epidemiology

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## Abstract

Much of the existing theory for the evolutionary biology of infectious diseases uses an invasion analysis approach. In this Ideas and Perspectives article, we suggest that techniques from theoretical population genetics can also be profitably used to study the evolutionary epidemiology of infectious diseases. We highlight four ways in which population-genetic models provide benefits beyond those provided by most invasion analyses: (i) they can make predictions about the rate of pathogen evolution; (ii) they explicitly draw out the mechanistic way in which the epidemiological dynamics feed into evolutionary change, and thereby provide new insights into pathogen evolution; (iii) they can make predictions about the evolutionary consequences of non-equilibrium epidemiological dynamics; (iv) they can readily incorporate the effects of multiple host dynamics, and thereby account for phenomena such as immunological history and/or host co-evolution.

## Keywords

Antigenic evolution, drug resistance, epidemiology, infectious diseases, parasite, pathogen, virulence.

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## INTRODUCTION

One of the most challenging aspects of theoretical evolutionary ecology is accounting for the feedback between ecological and evolutionary processes. The study of pathogen evolution provides an excellent example of the potential complexities of such feedbacks (Dieckmann *et al.* 2002). The epidemiological dynamics of a disease determine, in part, the way that natural selection acts on the pathogen population. At the same time, the resulting evolutionary change in the pathogen will affect the nature of the epidemiological dynamics. Understanding such feedbacks, and teasing apart how various external factors affect these processes, is one of the central goals of the evolutionary epidemiology of infectious disease.

Various theoretical frameworks have been developed in evolutionary ecology to account for eco-evolutionary feedbacks, ranging from relatively simple single-locus genetic models (Charlesworth 1971; Roughgarden 1971) to quantitative-genetic models (Slatkin 1980; Pease *et al.* 1989) to various types of evolutionary invasion analyses (Lawlor & Maynard Smith 1976; Reed & Stenseth 1984; Vincent *et al.* 1993; Dieckmann & Law 1996; Geritz *et al.* 1998; for reviews see Taper & Case 1992; Abrams 2001;

Day 2005; Waxman & Gavrillets 2005). Despite this variety of approaches in evolutionary ecology, many studies of evolutionary epidemiology tend to employ invasion analyses, particularly those studies of virulence evolution. This approach simplifies epidemiological and evolutionary feedbacks by assuming that the epidemiological dynamics are very fast relative to evolutionary change (Frank 1996; Dieckmann *et al.* 2002). This implies that the feedback from evolutionary change through the epidemiological dynamics, and back to evolution, is effectively instantaneous. Such theory is mostly geared toward making predictions about long-term evolutionary equilibria.

The focus on long-term equilibria in invasion analyses is where the approach gains one of its strengths. By employing a separation of timescales between evolutionary and epidemiological processes, one can often make analytical progress with models that might otherwise be intractable. At same time, however, important information is lost when separating timescales. In this Ideas and Perspectives article, we suggest that the invasion analysis approach can be profitably combined with a population-genetics (PG) approach that relaxes this assumption, and that treats the ecological/epidemiological side of the

feedback on equal footing with the evolutionary side. Although sometimes analytically less tractable than an invasion analysis, we believe this approach offers at least four advantages: (i) it allows one to make predictions about the rate of pathogen evolution; (ii) it explicitly draws out the mechanistic way in which the epidemiological dynamics feed into evolutionary change, and thereby provides new insights into pathogen evolution; (iii) it allows one to model the evolutionary consequences of non-equilibrium epidemiological dynamics; (iv) it allows one to readily model the dynamics of multiple host types, and thereby to account for phenomena such as immunological history and/or host co-evolution.

In the remainder of this article, we begin by introducing a simple epidemiological model that will be used to draw out our main points. Section 3, then, briefly describes the invasion analysis and the population-genetic approaches in the context of this model. Section 4 goes on to illustrate each of the four claims made above, and section 5 provides a summary and brief discussion.

## A SIMPLE EPIDEMIOLOGICAL SETTING

The core elements of the points we wish to emphasize are best illustrated with a simple SIS epidemiological model (Hethcote 2000):

$$\frac{dS}{dt} = \theta - \mu S - \beta SI + \gamma I \quad (1a)$$

$$\frac{dI}{dt} = \beta SI - (\mu + v + \gamma)I \quad (1b)$$

The parameter  $\theta$  is the immigration rate of susceptible hosts,  $\mu$  is their per capita background mortality rate,  $v$  is their increased mortality rate due to infection (i.e. virulence),  $\gamma$  is their rate of recovery from infection and  $\beta$  is the transmission rate. Model (1) has two equilibria, one with the pathogen absent ( $\hat{S} = \theta/\mu, \hat{I} = 0$ ) and one with it present

$$\hat{S} = \frac{\mu + v + \gamma}{\beta}, \quad \hat{I} = \frac{\theta}{\mu + v} - \frac{\mu}{\beta} \cdot \frac{\mu + v + \gamma}{\mu + v}.$$

The disease-free equilibrium is always biologically feasible, whereas the endemic equilibrium is feasible if, and only if,  $R_0 > 1$  where  $R_0 = (\theta/\mu)[\beta/(\mu + v + \gamma)]$ . Furthermore, it can be shown that, when  $R_0 > 1$ , the endemic equilibrium is globally asymptotically stable; otherwise, the disease-free equilibrium is globally asymptotically stable (Korobeinikov & Wake 2002). In all analyses that follow, transmission rate,  $\beta$ , virulence,  $v$  and recovery rate,  $\gamma$ , are potentially dependent on pathogen strain. We restrict attention to strains for which  $R_0 > 1$ .

## THE TWO APPROACHES IN EVOLUTIONARY EPIDEMIOLOGY

### An invasion analysis approach

An invasion analysis begins by supposing that a single pathogen strain is present at its endemic steady state. A small number of individuals carrying a second strain are then introduced, and one determines if this second strain can increase in number. Of particular interest are strains that, once present at an endemic equilibrium, can resist invasion by all rare mutant strains. Such strains are termed evolutionarily stable (ES; Otto & Day 2007).

Under the assumption that an already infected host cannot be infected by another parasite strain (i.e. no multiple infections) a global invasion analysis shows that the strain with the largest value of  $R$  is evolutionarily stable, where  $R \equiv R_0 \cdot (\mu/\theta) = \beta/(\mu + v + \gamma)$ . Thus, strains with high transmission and low virulence and/or recovery are 'best'. For some pathogens, genotypes with a high transmission rate tend also to induce a high mortality rate (Anderson & May 1982; Ebert 1994; Ebert & Mangin 1997; Lipsitch & Moxon 1997; Mackinnon & Read 1999; Messenger *et al.* 1999). If recovery rate is independent of strain type, such tradeoffs are most simply accounted for by supposing that transmission rate is an increasing function of virulence. In this case, we then seek the level of virulence,  $v$ , that maximizes  $R = \beta(v)/(\mu + v + \gamma)$ . As long as the function  $\beta(v)$  increases at a diminishing rate (i.e.  $d^2\beta/dv^2 < 0$ ),  $R$  will be maximized at an intermediate level of virulence.

### A population-genetic approach

Population-genetic techniques have recently been adapted to model the dynamics of the frequency of different pathogen strains, in conjunction with the dynamics of host population size (Day & Proulx 2004; Day & Gandon 2006). In the context of model (1), the approach begins by first extending the model to allow for  $n$  pathogen strains. One then changes variables to model the frequencies of the strains, along with the total number of susceptible and infected individuals. Defining  $I_T = \sum_i I_i$  as the total number of infected individuals, and  $q_i = I_i/I_T$  as the frequency of strain  $i$ , we obtain

$$\frac{dq_i}{dt} = q_i(r_i - \bar{r}) - \eta q_i + \eta \sum_j m_{ji} q_j \quad (2a)$$

$$\frac{dS}{dt} = \theta - \mu S - S\bar{\beta}I_T + \gamma I_T \quad (2b)$$

$$\frac{dI_T}{dt} = S\bar{\beta}I_T - (\mu + \bar{v} + \gamma)I_T \quad (2c)$$

where  $r_i = S\beta_i - v_i - \gamma$  is the fitness of strain  $i$ , and the overbars denote an expectation over the distribution of pathogen strains; i.e.  $\bar{x} = \sum_i q_i x_i$ . (For simplicity, we have also assumed that recovery rate is independent of strain type. Analogous results are obtained in the more general case; Day & Proulx 2004; Day & Gandon 2006). We have also added 'mutation' terms in eqn (2a). When pathogen mutations arise, they do so in infected hosts. This creates a host harbouring more than one strain of pathogen. We simplify this process by assuming that such mutations immediately die out or supplant the original strain. 'Mutation' therefore represents a change from one genotype of infection to another, and occurs at rate  $\eta$ . The parameter  $m_{ji}$  is the probability that, given such a change, an infection of genotype  $j$  changes to one of genotype  $i$  (Day & Gandon 2006).

Equation (2) separates the evolutionary dynamics (eqn 2a) from the epidemiological dynamics (eqns 2b,c). At this stage it is then often useful to derive a form of Price's equation from eqn (2a) that tracks the mean value of any character of interest (Price 1970). For example, if we are interested in the mean level of virulence and transmission, we can differentiate  $\bar{v} = \sum_i q_i v_i$  and  $\bar{\beta} = \sum_i q_i \beta_i$  with respect to time, using eqn (2a) (Day & Gandon 2006):

$$\frac{d\bar{v}}{dt} = \text{cov}(v_i, r_i) - \eta(\bar{v} - \bar{v}_m), \quad (3a)$$

$$\frac{d\bar{\beta}}{dt} = \text{cov}(\beta_i, r_i) - \eta(\bar{\beta} - \bar{\beta}_m). \quad (3b)$$

Here,  $\bar{x}_m = \sum_{i,j} x_i m_{ji} q_j$  is the average value of trait  $x$  among all new 'mutations'. The average value of any trait changes in a direction given by the sign of the covariance between the trait and fitness, plus any directional change owing to mutation (Price 1970; Day & Gandon 2006).

To gain more insight into the evolution of transmission rate and virulence, the covariance terms in eqn (3) can be expanded as

$$\frac{d\bar{v}}{dt} = S\sigma_{v\beta} - \sigma_{vv} - \eta(\bar{v} - \bar{v}_m) \quad (4a)$$

$$\frac{d\bar{\beta}}{dt} = S\sigma_{\beta\beta} - \sigma_{\beta v} - \eta(\bar{\beta} - \bar{\beta}_m) \quad (4b)$$

where  $\sigma_{xy}$  is the covariance between  $x$  and  $y$  across pathogen strains. Equation (4) can also be written in matrix notation as:

$$\begin{pmatrix} \frac{d\bar{v}}{dt} \\ \frac{d\bar{\beta}}{dt} \end{pmatrix} = \mathbf{G} \begin{pmatrix} S \\ -1 \end{pmatrix} - \eta \begin{pmatrix} \bar{v} - \bar{v}_m \\ \bar{\beta} - \bar{\beta}_m \end{pmatrix} \quad (5)$$

where  $\mathbf{G}$  is the genetic (co)variance matrix and  $(S-1)^T$  is the selection gradient.

Equation (5) is analogous to quantitative genetics models (Lande 1976; Lande & Arnold 1983; Day & Proulx 2004), but it does not rely on Gaussian distributions of strain phenotypes, nor on an assumption of small variance. The product of  $\mathbf{G}$  with the selection gradient in eqn (5) is an exact description of the effect of natural selection on the average level of virulence and transmission. Natural selection favours reduced virulence with a strength of  $-1$ . On the other hand, natural selection favours an increased transmission rate with strength proportional to the density of susceptible hosts,  $S$ . Thus, for example, direct selection always drives virulence downward at a strength of  $-1$ , mediated by the genetic variance in virulence. At the same time, indirect selection pulls virulence upward with a strength proportional to the density of susceptible hosts, mediated by the genetic covariance between transmission and virulence. An analogous interpretation can be given to the evolutionary dynamics of transmission.

In the short-term, the  $\mathbf{G}$  matrix and the selection gradient can be assumed constant, and eqn (5) used to predict the direction and the speed of evolution. In the longer-term, the epidemiological dynamics given in eqns (2b,c) allow one to track changes in the selection gradient. Change in the  $\mathbf{G}$  matrix is more difficult to track since it depends on the selection gradient, the mutation rate, and of the effect of mutations. Additional assumptions regarding the distribution of strain frequencies can be used to derive dynamical equations for these variance components (Day & Proulx 2004).

## RESULTS

We now proceed to illustrate how the PG approach addresses each of the four points raised in the introduction.

### Predicting the speed of pathogen evolution

In most invasion analyses, one focuses on predicting the endpoint of evolution, whereas the PG approach also allows one to predict the transient evolutionary dynamics. This can be useful when making theoretical predictions about experimental manipulations because most such studies are conducted on relatively short time scales. Such predictions can also be valuable when attempting to forecast the effects of public health interventions.

As a simple example, consider the coupled evolutionary and epidemiological dynamics of the spread of drug resistant mutants. Suppose the host-parasite system has reached an endemic equilibrium, and we begin administering a new drug to all infected individuals. This drug results in a higher recovery rate from infection by hosts infected with the wild type pathogen. Some mutant strains are able to resist the drug, however, and we consider two types of resistance: (i) a

**Table 1** Parameter values used in Fig. 1. Note that  $R = R_0(\mu/\theta)$ .

	Without drug				With drug			
	$\beta$	$\nu$	$\gamma$	$R$	$\beta$	$\nu$	$\gamma$	$R$
Wild type	$7.25 \times 10^{-4}$	0	26	$2.78 \times 10^{-5}$	$7.25 \times 10^{-4}$	0	58	$1.25 \times 10^{-5}$
Transmission variant	$3.625 \times 10^{-4}$	0	26	$1.39 \times 10^{-5}$	$3.625 \times 10^{-4}$	0	26	$1.39 \times 10^{-5}$
Virulence variant	$7.25 \times 10^{-4}$	27	26	$1.37 \times 10^{-5}$	$7.25 \times 10^{-4}$	27	26	$1.37 \times 10^{-5}$

*transmission* variant that pays a cost of drug resistance through reduced transmission; (ii) a *virulence* variant that pays a cost of drug resistance through increased virulence (Table 1).

Before the drug is introduced, the wildtype strain has the highest value of  $R$ , and an invasion analysis correctly predicts that it will exclude the other strains (Fig. 1). The drug resistant variants are maintained at low frequency via mutation-selection balance, and eqn (2) can be used to find their equilibrium frequencies.

Once the drug is introduced the strain hierarchy is altered, and the wildtype has the lowest value of  $R$  (Table 1). A numerical simulation confirms that the wildtype decreases in frequency, but the outcome of the competition between two drug resistant variants is not simply governed by the  $R$ -values (Fig. 1). Initially, the drug resistant variant with the lower  $R$  (the virulent variant) reaches the highest frequency, and then eventually it gives way to the variant with the highest  $R$  (the transmission variant). Thus, although the invasion analysis correctly predicts which drug resistant

variant will prevail in the long term, it provides no information about the short-term evolutionary dynamics, nor any information about the rate at which resistant types spread.

The population-genetic approach embodied by eqn (2a) provides an explanation for the transient competitive advantage enjoyed by the virulent variant. Using  $q^*$  to denote the frequency of a focal variant and neglecting mutation, eqn (2a) is

$$\frac{dq^*}{dt} = \sigma s^* \quad (6)$$

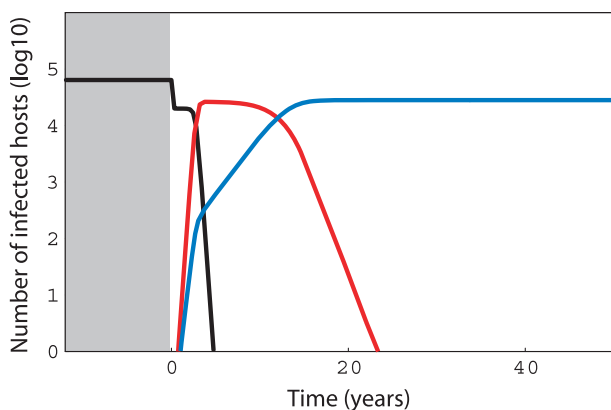
where  $\sigma = q^*(1-q^*)$  is the genetic variance with respect to the focal strain, and  $s^* = r^* - \tilde{r}$  is the selection co-efficient of this variant. Specifically,  $r^*$  is the variant's fitness,  $\beta^* S - \mu - \nu^* - \gamma^*$ , and

$$\tilde{r} = \frac{\sum_{j \neq *} q_j r_j}{1 - q^*}$$

is the average fitness of all other strains. This equation tells us that the speed of evolution depends on the product of the strain variance and the focal variant's selection co-efficient.

Shortly after the drug is introduced, the two drug resistant variants are rare, and the density of susceptible hosts is governed by the characteristics of the wild type. The drug is very efficient at clearing infections by the wild type strain, and thus it increases the number of susceptible hosts,  $S$ . Under these conditions, the virulent variant (with its higher transmission rate) has a higher fitness than the transmission variant. It pays the cost of resistance in terms of virulence rather than transmission, and is therefore, best able to exploit this transient increase in susceptible hosts. In the long-term, however, the number of susceptible hosts eventually decreases again once drug resistance has begun to spread throughout the population. This then tips the balance towards the transmission variant, and ultimately it dominates the population.

This example illustrates a twofold benefit of a population-genetic approach. First, eqn (2a) and thus eqn (6) can be used to predict the speed of evolution as both  $\sigma$  and  $s^*$  can be derived from the parameters in Table 1. Second, this



**Figure 1** Numerical simulation showing the outcome of competition between different pathogen variants (black: wild type, red: virulent variant, blue: transmission variant) before (in gray) and after (in white) the use of a drug (drug starts to be used at  $t = 0$ ). We allow the three strains to emerge by mutation ( $\eta = 10^{-5}$ ). Total size of the population is assumed to remain fixed (dead hosts are replaced by susceptible ones) and equal to  $10^5$ , and parameter values are given in Table 1 with  $\mu = 0.02$ .

formalism allows one to follow the evolution of the parasite population when it is away from its endemic equilibrium. Such transient dynamics can be counter-intuitive, and can have important consequences. For instance, the drug-induced transient evolutionary increase in virulence seen in this example would be missed by an invasion analysis. Similar phenomena can also occur in more complex situations, where the host population is heterogeneous, or where only a fraction of the population is treated (see Gandon & Day 2007, for examples, involving vaccination).

### What novel insights do we gain from a population-genetic approach?

The PG formulation is clearly beneficial when we are interested in short-term evolutionary predictions, but it can also yield novel insights into the long-term evolution of pathogens. To illustrate this point, we consider two questions that have previously been addressed with invasion analyses: What is the effect of host mortality on virulence evolution, and what is the effect of vertical versus horizontal transmission on virulence evolution? For each, we consider how a population-genetic formulation can aid in designing experimental tests of predictions, and thereby how it can enhance our understanding of virulence evolution.

#### *Effect of host mortality*

There are many invasion analyses for the effects of host mortality on virulence evolution (Anderson & May 1982; Sasaki & Iwasa 1991; Kakehashi & Yoshinaga 1992; Lenski & May 1994; Ebert & Weisser 1997). Although there are exceptions (Williams & Day 2001; Day 2002), the vast majority of these predict that higher host mortality rates lead to the evolution of higher pathogen virulence. This prediction is readily derived from model (1). Under the simplifying assumption that recovery rate is independent of strain type, evolution maximizes  $\beta(v)/(\mu + v + \gamma)$ , and the first derivative condition for a maximum is

$$\frac{d\beta}{dv} = \frac{\beta(v)}{\mu + v + \gamma}. \quad (7)$$

Larger values of  $\mu$  decrease the right-hand side of (eqn 7). As  $d\beta/dv$  decreases with increasing  $v$  (i.e.  $d^2\beta/dv^2 < 0$ ), larger values of  $\mu$  makes the value of  $v$  at which equality is attained in (eqn 7) larger. One interpretation of this is that, with higher host mortality, future reproduction (of the pathogen) is less valuable thereby tipping the balance towards greater current exploitation and thus virulence.

How might this prediction be tested experimentally? Ideally one would have control populations of a host and its parasite, and treatment populations in which a higher host mortality rate is induced. But how, precisely, do we accomplish this treatment? Consider the following three

possibilities: (a) we periodically remove a fraction of hosts; (b) we periodically remove a fraction of hosts and replace them with susceptible individuals; (c) we periodically remove a fraction of hosts and replace them with randomly selected hosts from a parallel control population (thus some of the replacement individuals are susceptible and others infected). One can readily imagine any of these three protocols being implemented in many experimental systems, and in fact protocol (b) is exactly that used in an influential study by Ebert & Mangin (1997).

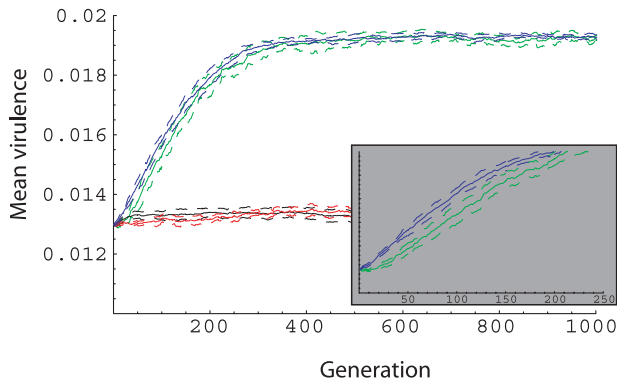
Protocol (a) is the simplest, but it confounds heightened host mortality rate with a change in host population size. Protocol (b) maintains the same heightened host mortality rate as protocol (a) and it controls host population size; however, it confounds the heightened mortality with a change in host population composition, because some of the removed hosts that were infected are replaced with susceptible hosts. Finally, protocol (c) maintains the same heightened host mortality rate, and controls both host population size and composition. Interestingly, one might also interpret protocol (b) as an introduction of recovery from infection (Gandon *et al.* 2001), and protocol (c) as an introduction of migration between separate populations, rather than manipulations of mortality.

Each of these protocols has its merits, and we do not wish to advocate one over another. But individual-based simulations reveal that these protocols yield very different evolutionary outcomes (Fig. 2). Protocol (a) yields an evolutionary increase in virulence, as does protocol (b), although protocol (b) causes evolution to occur slightly more quickly (Fig. 2 inset). Protocol (c), on the contrary, results in no difference between control and treatment populations. These findings are initially surprising since the hosts in all protocols suffer the same heightened mortality rate and thus reduced expected lifespan. Consequently, together these experimental results suggest that it is not mortality *per se* that governs virulence evolution. The PG equation for virulence evolution (eqn 4a) provides insight into these results. Neglecting mutation, we have:

$$\frac{d\bar{v}}{dt} = S\sigma_{v\beta} - \sigma_{vv}. \quad (8)$$

Interestingly, eqn (8) shows that *host mortality does not directly affect the dynamics of virulence evolution* at all. Rather, it has only an indirect effect through either the density of susceptible hosts,  $S$ , or through the genetic parameters. We do not expect the above manipulations of host mortality to significantly alter the genetic parameters, so any effect must therefore be mediated through the epidemiological dynamics of  $S$ .

At first glance this seems at odds with the invasion analysis, but this apparent inconsistency is resolved by recalling that the invasion analysis assumes that the



**Figure 2** Results of simulations for the experimental manipulations of host mortality as proposed in the text (details and notation in Appendix). Two pathogen strains are modeled with transmission, virulence, and clearance rates:  $\tau_1 = 0.7$ ,  $\alpha_1 = 0.01, c_1 = 0.01$  and  $\tau_2 = 0.9$ ,  $\alpha_2 = 0.02, c_2 = 0.01$ . There is no vertical transmission ( $f = 0$ ) and a probability of mortality of 30% is experimentally imposed every 6th generation for all treatments but the control. Solid lines are the average of 10 replicates, and dashed lines are the 95% confidence intervals. Black = control. Green = protocol (a) – mortality with no replacement. Blue = protocol (b) – mortality and replacement with  $S$ . Red = protocol (c) – mortality and replacement with parallel stock. Inset is a close-up of protocols (a) and (b) over a shorter time scale, to reveal transient differences.

population is always at epidemiological equilibrium. In this case, we have  $S = (\mu + \bar{v} + \gamma)/\beta$ , and eqn (8) becomes

$$\frac{d\bar{v}}{dt} = \frac{\mu + \bar{v} + \gamma}{\beta} \sigma_{v\beta} - \sigma_{vv}. \tag{9}$$

From (eqn 9) we see that, in an invasion analysis, an increase in host mortality is assumed to indirectly result in an immediate increase in the density of susceptible hosts. This increases the strength of selection for higher transmission rate, and thereby drags virulence to higher values through its positive genetic correlation with transmission. At evolutionary equilibrium  $d\bar{v}/dt = 0$ , and eqn (9) gives

$$\frac{\sigma_{v\beta}}{\sigma_{vv}} = \frac{\bar{\beta}}{\mu + \bar{v} + \gamma}, \tag{10}$$

which is the population-genetic analogue of eqn (7). This illustrates that, in spite of the seeming independence of fitness with regard to the density of susceptible hosts in eqn (7), it is this density that actually indirectly selects for higher virulence.

Importantly, in the above experiments, the epidemiological feedback implicit in eqn (7) need not occur. Protocol (a), by not controlling host density, allows for such an epidemiological feedback, although it is not instantaneous. Protocol (b) does not allow for the feedback, but it instead artificially enhances the density of susceptible hosts via its

alteration of host population composition. The increase in the density of susceptibles is thus instantaneous in protocol (b) and delayed in protocol (a). This explains the short-term evolutionary dynamics seen in the inset to Fig. 2. The faster increase in density of susceptibles in protocol (b) drives the increase in virulence more quickly. In the long term, however, both protocols yield the same evolutionary outcome because, at equilibrium, both protocols lead to the same density of susceptible hosts (although the density of infected hosts will be different). Protocol (c), on the other hand, keeps  $S$  constant while increasing host mortality, and therefore results in no evolutionary change, as predicted from eqn (8).

The indirect dependence of virulence on mortality also helps to reconcile evolutionary epidemiology with verbal predictions that higher access to susceptible hosts (e.g. via increased air or water born transmission) should select for higher virulence (Ewald 1994). This argument is consistent with eqn (8) but misses the epidemiological feedback following the increase in transmission. Ultimately, higher transmissibility results in a decrease in the density of susceptibles, in some cases resulting in no net change in the evolutionary outcome (see also Lenski & May 1994).

*Vertical vs. horizontal transmission and virulence evolution*

Another prediction from theoretical evolutionary epidemiology is that parasites with vertical transmission (VT) should evolve to be less virulent than parasites with horizontal transmission (HT; Nowak 1991; Ebert & Herre 1996; Frank 1996; Lipsitch *et al.* 1996; Gandon 2004). The reproductive success of vertically transmitted pathogens is tied to host reproduction, and therefore they have a greater stake in not harming their hosts. Thus, we might expect experiments that increase the occurrence of vertical transmission relative to horizontal transmission to select for decreased virulence. Two experimental studies using phage and their bacterial hosts have verified this prediction (Bull *et al.* 1991; Messenger *et al.* 1999).

In principle, the VT : HT ratio can be increased by either increasing VT, decreasing HT, or a combination of the two. The above-mentioned experimental studies have both altered this ratio through changes in both transmission modes, presumably because this is the most feasible experimental design. But would it make a difference to the evolutionary outcome if we could increase this ratio by varying only one or the other transmission mode? As with the experiment on host mortality rate, it is critical that we carefully account for the way in which the epidemiological dynamics generate selection on virulence, and how these dynamics are affected by the experimental protocol.

Model (1) can be extended to allow for vertical transmission by replacing  $\theta$  with the term  $a(N)S + b(N)(1-f)I$ , where  $N$  is the total population size,  $a(N)$  and  $b(N)$  are the

per capita birth rates specific to susceptible and infected hosts, and  $f$  is the probability of vertical transmission. We then also append the term  $b_i(N)f$  to the equation for the dynamics of  $I$ . Supposing that all strains endure the same recovery rate, we can again derive eqn (3a) but with the fitness of pathogen strain  $i$  now given by  $r_i = b_i(N)f + \beta_i - \mu - v_i - \gamma$ . The only change from the previous expression for fitness is the inclusion of an extra term that represents the production of new infections through vertical transmission. Neglecting mutation, the analogue of eqn (8) can then be written as

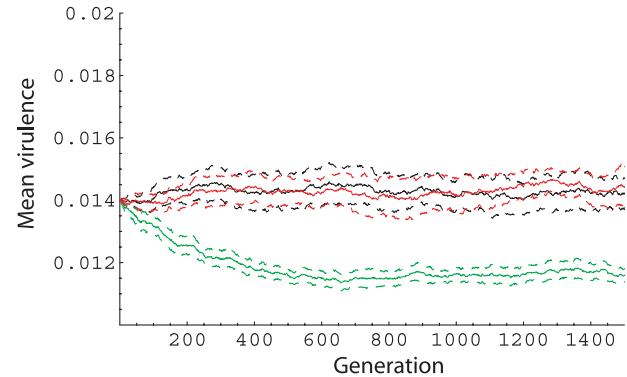
$$\frac{d\bar{v}}{dt} = \text{cov}(b_i(N), v_i) + S\sigma_{\beta v} - \sigma_{vv}. \quad (11)$$

Again virulence evolves due to the same factors as before [the last two terms in eqn (11)], but it now also potentially evolves as a result of indirect selection on the rate of reproduction of infected hosts, and the genetic covariance between this and virulence [the first term in eqn (11)]. The majority of considerations of the effects of vertical transmission implicitly ignore any potential covariance between host reproduction rate and virulence (when defined as a mortality rate). In this case, host birth rate is independent of pathogen strain, although infected hosts might still have a lower birth rate than susceptible hosts. Then eqn (11) simplifies to exactly that given by eqn (8). In other words, *the presence of vertical transmission then has no direct effect on the evolutionary dynamics of virulence*. Thus experimental manipulations of the relative amounts of vertical vs. horizontal transmission will induce evolutionary changes in virulence, only if these indirectly alter the density of susceptible hosts via an epidemiological feedback, or if they alter the genetic parameters.

The experimental protocols used in the above-mentioned experimental studies both increased the VT : HT ratio, in part by preventing HT at times. This thereby removes any genetic covariance between virulence and transmission at these times. As predicted by eqn (8), the result was then an evolutionary decrease in virulence. One can also use individual-based simulations to determine the experimental outcome if instead the VT : HT ratio is increased while holding the degree of HT constant (Appendix). As predicted by eqn (8) this manipulation of the VT : HT ratio leaves virulence unchanged (Fig. 3).

### What is the effect of non-equilibrium epidemiology on pathogen evolution?

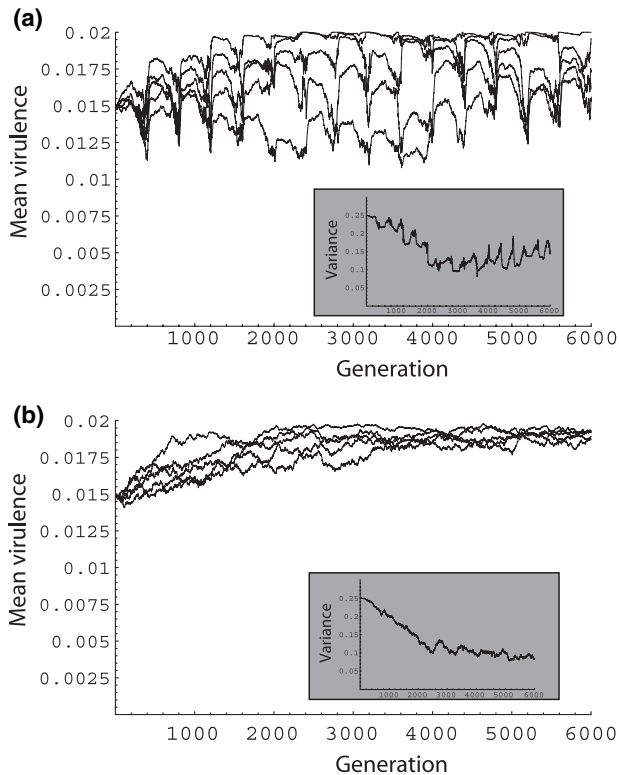
Another circumstance in which a PG formulation can be useful arises when there are persistent non-equilibrium epidemiological dynamics. Many infectious diseases are characterized by such dynamics, either because of the inherent nonlinearities in the epidemiology and/or seasonal



**Figure 3** Results of simulations for the experimental manipulations of transmission mode (details and notation in Appendix). Two pathogen strains are modeled with transmission, virulence, and clearance rates:  $\tau_1 = 0.79$ ,  $\alpha_1 = 0.01, \epsilon_1 = 0.1$  and  $\tau_2 = 0.86$ ,  $\alpha_2 = 0.02, \epsilon_2 = 0.1$ . Vertical transmission is 100% ( $f = 1$ ). Solid lines are the average of 20 replicates, and dashed lines are the 95% confidence intervals. Black = control. Red = protocol (a) – reduced VT, constant HT transmission. Every 2 days all infections generated by vertical transmission on that day are removed. These are then replaced with new horizontally derived infections, choosing the strains used in the replacement in proportion to their relative abundance at the beginning of that day (so as not to create an evolutionary bias with this replacement). Green = protocol (b) – reduced HT, constant VT. Every 2 days all infections generated by horizontal transmission on that day are removed. These are then replaced with new vertically derived infections, choosing the strains used in the replacement in proportion to their relative abundance at the beginning of that day. Both replacement protocols ensure that the number of susceptible and infected hosts is unchanged by the manipulation, with protocol (b) removing the genetic covariance between transmission and virulence every 2 days and protocol (a) leaving the genetic parameters unchanged.

forcing. Furthermore, in many experimental systems (e.g. phage and bacteria) the culturing protocols themselves induce non-equilibrium epidemiological dynamics. In these cases, we might expect non-equilibrium evolutionary dynamics, making the equilibrium assumptions of invasion analyses inappropriate. An approach that treats the epidemiological and evolutionary dynamics on arbitrary time-scales is necessary to capture such phenomena.

As a simple example consider a pathogen, such as directly transmitted childhood infectious disease, for which contact rates are high during school terms and low outside of these times. In this case, the transmission co-efficient,  $\beta$ , will oscillate seasonally, and eqn (4) predicts that the mean level of virulence should oscillate evolutionarily as well. Fig. 4 presents results of simulations that compare the evolutionary dynamics of mean virulence when contact rates fluctuate seasonally (Fig. 4a) vs. when they are constant (Fig. 4b). As can be seen, fluctuating contact rates not only result in



**Figure 4** Results of simulations using the model from the control population (details and notation in Appendix). Two pathogen strains are modeled with transmission, virulence, and clearance rates:  $\tau_1 = 0.7$ ,  $\alpha_1 = 0.01$ ,  $c_1 = 0.01$  and  $\tau_2 = 0.9$ ,  $\alpha_2 = 0.02$ ,  $c_2 = 0.01$ . There is no vertical transmission ( $f = 0$ ). (a) Five replicate populations in which the contact rate between individuals oscillates every 200 generations between  $\xi = 0.00001$  and  $\xi = 0.00008$ . (b) Five replicate populations in which the contact rate is constant at  $\xi = 0.0000283$  (the geometric mean of 0.00001 and 0.00008). Inset figures reveal the average genetic variance within each replicate over time.

corresponding fluctuations in the evolutionary dynamics, but they also tend to have interesting effects on the variation in evolutionary outcomes between replicate populations (Fig. 4) and on the maintenance of genetic variance within replicate populations (Fig. 4 insets).

Interestingly, the study of virulence evolution by Messenger *et al.* (1999) involved a protocol with a similar non-equilibrium effect as it alternately imposed vertical and horizontal transmission. Using the above results as a guide, we would not only expect this to induce non-equilibrium evolutionary dynamics, but to induce considerable variation among replicates as well. Ebert & Bull (2003) have indeed suggested that the study by Messenger *et al.* (1999) had small effect sizes and extensive variation, and the above results might therefore provide a plausible explanation for these findings. Further, stochastic extensions of the PG

approach would allow one to generate predictions of the between replicate variation expected in this sort of study. They would also allow one to incorporate the effects of stochastic strain extinction for situations in which this is likely to be important (e.g. when the number of infections is small).

### Incorporating host heterogeneity into evolutionary epidemiology

Although most of the above presentation has focused on simple evolutionary scenarios in homogeneous host populations, another strength of the PG approach is its ability to deal with the dynamics of multiple host types. Invasion analysis can be extended to study the evolution of parasites infecting multiple hosts, but this approach relies on the assumption that the community of hosts has reached a stable equilibrium (Gandon 2004). The PG approach allows one to analyse evolutionary dynamics away from such equilibria. For example, there is an extensive and growing body of literature on antigenic evolution of pathogens in response to a temporally variable host heterogeneity (both genetic and immunological heterogeneity). The PG approach can be used to meld these kinds of models with models for the evolution of pathogen virulence and transmission.

As a simple example, consider extending model (1) to incorporate host heterogeneity (either genetic or immunological). Let us suppose that transmission of the pathogen involves the product of two type-specific parameters:  $\beta_{ij}$ , the production rate of parasite strain  $i$  when infecting a host of type  $j$ , and  $\sigma_{ij}$ , the probability of infection by parasite of strain  $i$  in host of type  $j$ , given that exposure occurs. Neglecting mutation, and using  $S_j$  to denote the number of susceptible hosts of type  $j$ ,  $I_{ij}$  to denote the number of hosts of type  $j$  that are infected by pathogens of strain  $i$ , and assuming that virulence and recovery rate are affected by both host type and pathogen strain, we have

$$\frac{dS_j}{dt} = F_j(S_k, I_{lk}) \tag{12a}$$

$$\frac{dI_{ij}}{dt} = \sigma_{ij} S_j \sum_k \beta_{ik} I_{ik} - (\mu + v_{ij} + \gamma_{ij}) I_{ij}, \tag{12b}$$

where  $k$  runs over all host types, and  $l$  runs over all pathogen strains. The form of the functions  $F_j$  will be determined, in part, by whether the host heterogeneity is due to immunological history, and is thus a plastic trait, or if it is due to host genotype, and thus evolves.

We can again derive equations for the dynamics of strain frequency,  $q_i \equiv \sum_j I_{ij} / \sum_{ij} I_{ij}$ , obtaining an equation analogous to eqn (2a) (Gandon and Day, unpublished results):



$$\frac{dq_i}{dt} = (r_{i\bullet} - r_{\bullet\bullet})q_i \quad (13)$$

Here  $r_{i\bullet}$  is the fitness of parasite strain  $i$ , averaged over all host types, and is given by  $r_{i\bullet} = \beta_{i\bullet} S_e^i - (\mu + v_{i\bullet} + \gamma_{i\bullet})$ ,  $S_e^i \equiv \sum_j \sigma_{ij} S_j$  denotes the effective number of susceptible hosts from the perspective of parasite strain  $i$ , and  $v_{i\bullet}$  and  $\gamma_{i\bullet}$  are the virulence and recovery rates for strain  $i$ , averaged over all host types. Note that, because of host heterogeneity, each strain now experiences a different 'effective' density of susceptible hosts.

To complete the model, we also need equations for the dynamics of the host population, and the details of these will depend on the nature of host heterogeneity (Gandon and Day, unpublished results). Nevertheless, we can obtain insight into the pathogen side of the evolutionary story without making any assumptions about the form of host heterogeneity. For example, the evolutionary dynamics of mean virulence are now

$$\frac{dv_{\bullet\bullet}}{dt} = \text{cov}(\beta_{i\bullet} S_e^i, v_{i\bullet}) - \text{var}(v_{i\bullet}) - \text{cov}(v_{i\bullet}, \gamma_{i\bullet}) + \Delta v_{\bullet\bullet}^H. \quad (14a)$$

Equation (14a) reveals that mean virulence evolves as a result of four factors. First, mean virulence is pulled in the direction of the covariance between the virulence and the expected rate of transmissibility,  $\beta_{i\bullet} S_e^i$ . The expected rate of transmissibility of strain  $i$  is given by the product of expected production rate of propagules by that strain,  $\beta_{i\bullet}$  and the effective density of susceptible hosts for that strain,  $S_e^i$ . Second, virulence is always selected against at a strength proportion to the variance in virulence [just as in model (8)]. Third, mean virulence is pulled in a direction opposite to the sign of the covariance between virulence and recovery across strains, because strains that endure (on average) a high recovery rate are selected against. Finally, mean virulence also changes as a result of changes in the composition of the host population, denoted by the term  $\Delta v_{\bullet\bullet}^H$ . The specific form of this term will depend on whether host population composition changes as a result of evolution or as a result of plastic changes in immunological history. An equation analogous to eqn (14a) can also be derived for the dynamics of the average recovery rate.

In the same way, we can also look at antigenic evolution in this model. Most previous theoretical studies of antigenic evolution have centered on exploring the evolutionary dynamics of strain structure, as quantified by various metrics of diversity (e.g. Sasaki 1994; Andreasen *et al.* 1997; Gupta *et al.* 1998; Gog & Grenfell 2002; Gog & Swinton 2002; Gomes *et al.* 2002; Grenfell *et al.* 2004). Another approach that derives naturally from PG models is to describe antigenic evolution using a functional index of strain composition, such as the average level of infectivity of the

parasite in the population as a whole,  $\sigma_{\bullet\bullet}$ . In this way, the reference coordinates for describing strain structure are determined by the host population and therefore themselves evolve over time, giving an epidemiologically relevant measure of antigenic evolution. Using a derivation analogous to that for eqn (14a), we obtain:

$$\frac{d\sigma_{\bullet\bullet}}{dt} = \text{cov}(\beta_{i\bullet} S_e^i, \sigma_{i\bullet}) - \text{cov}(\sigma_{i\bullet}, v_{i\bullet}) - \text{cov}(\sigma_{i\bullet}, \gamma_{i\bullet}) + \Delta \sigma_{\bullet\bullet}^H. \quad (14b)$$

As with eqn (14a), the average level of infectivity evolves in response to four factors. First, the average infectivity is pulled in a direction given by the sign of the covariance between the infectivity of a strain and its expected rate of transmissibility. Second, average infectivity is pulled in a direction given by the opposite of the sign of the covariance between infectivity and virulence. For example, strains that are directly favoured as a result of their high transmissibility can be indirectly selected against if they tend also to induce a high level of virulence. Third, average infectivity is also pulled in a direction given by the opposite of the sign of the covariance between infectivity and recovery rate. For example, strains that are directly favoured as a result of their high transmissibility might also be indirectly favoured if they tend also to induce a low level of recovery. Finally, average infectivity also changes as a result of changes in the composition of the host population. For example, if the host population evolves in response to infection and/or it develops immunity to past infections, then this too results in a change in the average infectivity of parasite strains.

## SUMMARY AND DISCUSSION

A population-genetic perspective in theoretical evolutionary epidemiology can offer several benefits that complement those obtained from evolutionary invasion analyses. All of these benefits stem from the fact that a PG approach allows the evolutionary and epidemiological dynamics to each take place on arbitrary timescales. In this article, we have highlighted four potential benefits that we feel are particularly significant.

First, a PG perspective allows one to make quantitative predictions about the transient evolutionary dynamics of strain frequencies when the epidemiological dynamics are not at equilibrium. This is likely often the case for many infectious diseases, and our results demonstrate that transient evolutionary dynamics can be counterintuitive, and at odds with long-term predictions about evolutionary equilibria. This approach also allows one to make predictions about the speed of evolution, which can be valuable when studying the spread of drug- or vaccine-resistant pathogen strains (Gandon & Day 2007).

Second, a PG perspective provides novel insights into both the short- and the long-term evolution of pathogens. One reason for this is that the PG approach retains an explicit description of the mechanistic sources of selection on pathogen populations. Invasion analyses, on the contrary, typically dispense with these mechanistic details when assuming that the epidemiological dynamics are at equilibrium. Our results illustrate that the PG approach's focus on mechanism can lead to a more complete understanding of the factors governing pathogen evolution. This can have important consequences when attempting to test the theory experimentally (Figs 3 and 4). Similar findings apply to the evolutionary consequences of superinfection as well (Day & Proulx 2004; Day & Gandon 2006).

Third, a PG approach allows one to explore the evolutionary consequences of non-equilibrium epidemiological dynamics. Although there are ways to extend invasion analyses to deal with non-equilibrium dynamics (e.g. see Metz *et al.* 1992) these again employ a separation of timescales, and therefore, they eliminate the possibility of evolutionary change tracking short-term epidemiological fluctuations. As the results above illustrate, however, such rapid evolutionary responses can have important implications for our understanding of pathogen evolution and for the maintenance of pathogen diversity, both in response to natural and to experimental perturbations (Fig. 4).

Fourth, a PG approach provides a natural way to meld theory for the evolution of antigenic diversity with theory on the evolution of virulence and transmission. These two areas of research have developed, to a large extent, independently of one another. This is particularly evident in literature on the evolutionary consequences of vaccination, where some studies focus on virulence evolution and others focus on the evolution of escape mutants (Gandon & Day 2007). As the example above makes clear, a PG approach provides one route towards integrating these kinds of theories, and might thereby provide new insights into how antigenic evolution can affect the evolution of pathogen virulence and vice versa. Similarly, this approach could be used to merge host-parasite co-evolution into the evolutionary epidemiology framework. Many co-evolution models rely on the simplifying assumption that host and parasite population sizes are fixed. The PG approach provides a way to study simultaneously the co-evolutionary and epidemiological dynamics.

Aside from the benefits illustrated in the above examples, a PG approach also brings with it other interesting perspectives that warrant a brief mention. First, the concept of quasispecies has played an important role in discussions of viral evolution (Nowak 1992; Domingo 2002; Holmes & Moya 2002; Wilke 2003) and it is also intimately related to population-genetics theory (Moya *et al.* 2000; Bull *et al.* 2005; Wilke 2005). This concept has had

limited impact on the theory of virulence evolution, however, because most such theory is not framed in a population-genetic context. A PG approach can remedy this situation, and provides new suggestions for how pathogen virulence might evolve in the context of quasispecies (Day & Gandon 2006).

Although we have focused attention on simple epidemiological models that preclude multiple infections, it is also possible to extend the PG approach to allow for such effects. Interestingly, one approach for this involves the development of results analogous to Price's equation for evolutionary change (Price 1970, 1995). As with the formulation in eqn (3), there is a term involving the covariance between trait and fitness that arises from selection acting at the between-host level. Within-host selection arising from multiple infection, however, gives rise to an additional term that affects the direction of evolution (Day & Proulx 2004). This additional term is analogous to the 'transmission bias' term in the general form of Price's equation, and provides an interesting link between theoretical evolutionary epidemiology and the theory of multi-level selection (Frank 1998).

Finally, a PG approach readily allows one to make predictions about the rate of evolution of mean pathogen fitness. This is useful when determining whether a pathogen will die out or remain endemic. For example, in general we expect the dynamics of the number of infections for a model such as eqn (12) to obey the equation  $dI_T/dt = r_{\bullet\bullet} I_T$ . Therefore, the sign of the pathogen's expected fitness,  $r_{\bullet\bullet}$ , determines whether it remains extant ( $r_{\bullet\bullet} > 0$ ) or dies out ( $r_{\bullet\bullet} < 0$ ). An equation governing the dynamics of pathogen mean fitness can be readily derived, yielding an expression closely related to Fisher's Fundamental Theorem (Fisher 1930, 1958; Gandon and Day, unpublished results). In particular, this expression can be decomposed into two components first explicitly described by Price (Price 1972); a quantity  $\text{var}(r_{\bullet\bullet})$ , which is the variance in fitness among strains, and which reflects the direct effect of natural selection on the dynamics of mean fitness (Fisher 1930); and a quantity which represents what Fisher referred to as a degradation of the environment. In evolutionary epidemiology, a pathogen's environment is determined by the density and the composition of the host population. How this environment changes during the epidemiological and evolutionary dynamics will determine the rate of change of the environment, and thus will affect whether the balance between the effects of natural selection and the degradation of the environment comes out positive (pathogen persistence) or negative (pathogen extinction). Such models might offer valuable insights into how evolutionary change alters the ability of medical interventions to eradicate disease.

## REFERENCES

- Abrams, P.A. (2001). Modelling the adaptive dynamics of traits involved in inter- and intraspecific interactions: an assessment of three methods. *Ecol. Lett.*, 4, 166–175.
- Anderson, R.M. & May, R.M. (1982). Coevolution Of Hosts and Parasites. *Parasitology*, 85, 411–426.
- Andreasen, V., Lin, J. & Levin, S.A. (1997). The dynamics of cocirculating influenza strains conferring partial cross-immunity. *J. Math. Biol.*, 35, 825–842.
- Bull, J.J., Molineux, I.J. & Rice, W.R. (1991). Selection of benevolence in a host-parasite system. *Evolution*, 45, 875–879.
- Bull, J.J., Meyers, L.A. & Lachmann, M. (2005). Quasispecies made simple. *Plos Comput. Biol.*, 1, 450–460.
- Charlesworth, B. (1971). Selection in density regulated populations. *Ecology*, 52, 468–474.
- Day, T. (2002). On the evolution of virulence and the relationship between various measures of mortality. *Proc. R Soc. Lond. Ser. B Biol. Sci.*, 269, 1317–1323.
- Day, T. (2005). Modelling the ecological context of evolutionary change: déjà vu or something new? In: *Ecological Paradigms Lost: Routes of Theory Change* (eds Cuddington, K. & Beisner, B.E.). Academic Press, San Diego, USA, pp. 273–310.
- Day, T. & Gandon, S. (2006). Insights from Price's equation into evolutionary epidemiology. In: *Disease Evolution: Models, Concepts, and Data Analysis* (eds Feng, Zhilan, Dieckmann, Ulf & Levin, Simon A.). AMS, pp. 23–44.
- Day, T. & Proulx, S.R. (2004). A general theory for the evolutionary dynamics of virulence. *Am. Nat.*, 163, E40–E63.
- Dieckmann, U. & Law, R. (1996). The dynamical theory of coevolution: a derivation from stochastic ecological processes. *J. Math. Biol.*, 34, 579–612.
- Dieckmann, U., Metz, J.A.J., Sabelis, M.W. & Sigmund, K. (2002). *Adaptive Dynamics of Infectious Diseases: in Pursuit of Virulence Management*. Cambridge University Press, Cambridge, UK.
- Domingo, E. (2002). Quasispecies theory in virology. *J. Virol.*, 76, 463–465.
- Ebert, D. (1994). Virulence and local adaptation of a horizontally transmitted parasite. *Science*, 265, 1084–1086.
- Ebert, D. & Bull, J.J. (2003). Challenging the trade-off model for the evolution of virulence: is virulence management feasible? *Trends Microbiol.*, 11, 15–20.
- Ebert, D. & Herre, E.A. (1996). The evolution of parasitic diseases. *Parasitol. Today*, 12, 96–101.
- Ebert, D. & Mangin, K.L. (1997). The influence of host demography on the evolution of virulence of a microsporidian gut parasite. *Evolution*, 51, 1828–1837.
- Ebert, D. & Weisser, W.W. (1997). Optimal killing for obligate killers: the evolution of life histories and virulence of semelparous parasites. *Proc. R Soc. Lond. Ser. B Biol. Sci.*, 264, 985–991.
- Ewald, P.W. (1994). *Evolution of Infectious Disease*. Oxford University Press, Oxford.
- Fisher, R.A. (1930). *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.
- Fisher, R.A. (1958). *The Genetical Theory of Natural Selection*. 2nd edn. Dover, New York.
- Frank, S.A. (1996). Models of parasite virulence. *Q. Rev. Biol.*, 71, 37–78.
- Frank, S.A. (1998). *Foundations of Social Evolution*. Princeton University Press, Princeton, USA.
- Gandon, S. (2004). Evolution of multihost parasites. *Evolution*, 58, 455–469.
- Gandon, S. & Day, T. (2007). The evolutionary epidemiology of vaccination. *J. R Soc. Interface*, in press.
- Gandon, S., Jansen, V.A.A. & van Baalen, M. (2001). Host life history and the evolution of parasite virulence. *Evolution*, 55, 1056–1062.
- Geritz, S.A.H., Kisdi, É., Meszéna, G. & Metz, J.A.J. (1998). Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree. *Evol. Ecol.*, 12, 35–57.
- Gog, J.R. & Grenfell, B.T. (2002). Dynamics and selection of many-strain pathogens. *Proc. Natl Acad. Sci. USA*, 99, 17209–17214.
- Gog, J.R. & Swinton, J. (2002). A status-based approach to multiple strain dynamics. *J. Math. Biol.*, 44, 169–184.
- Gomes, M.G.M., Medley, G.F. & Nokes, D.J. (2002). On the determinants of population structure in antigenically diverse pathogens. *Proc. R Soc. B Biol. Sci.*, 269, 227–233.
- Grenfell, B.T., Pybus, O.G., Gog, J.R., Wood, J.L.N., Daly, J.M., Mumford, J.A. *et al.* (2004). Unifying the epidemiological and evolutionary dynamics of pathogens. *Science*, 303, 327–332.
- Gupta, S., Ferguson, N. & Anderson, R. (1998). Chaos, persistence, and evolution on strain structure in antigenically diverse infectious agents. *Science*, 280, 912–915.
- Hethcote, H.W. (2000). The mathematics of infectious diseases. *SIAM Rev.*, 42, 599–653.
- Holmes, E.C. & Moya, A. (2002). Is the quasispecies concept relevant to RNA viruses? *J. Virol.*, 76, 460–462.
- Takehashi, M. & Yoshinaga, F. (1992). Evolution of airborne infectious diseases according to changes in characteristics of the host population. *Ecol. Res.*, 7, 235–243.
- Korobeinikov, A. & Wake, G.C. (2002). Lyapunov functions and global stability for SIR, SIRS, and SIS epidemiological models. *Appl. Math. Lett.*, 15, 955–960.
- Lande, R. (1976). Natural selection and random genetic drift in phenotypic evolution. *Evolution*, 30, 314–334.
- Lande, R. & Arnold, S.J. (1983). The measurement of selection on correlated characters. *Evolution*, 37, 1210–1226.
- Lawlor, L.R. & Maynard Smith, J. (1976). The coevolution and stability of competing species. *Am. Nat.*, 110, 79–99.
- Lenski, R.E. & May, R.M. (1994). The evolution of virulence in parasites and pathogens – reconciliation between 2 competing hypotheses. *J. Theor. Biol.*, 169, 253–265.
- Lipsitch, M. & Moxon, E.R. (1997). Virulence and transmissibility of pathogens: what is the relationship? *Trends Microbiol.*, 5, 31–37.
- Lipsitch, M., Siller, S. & Nowak, M.A. (1996). The evolution of virulence in pathogens with vertical and horizontal transmission. *Evolution*, 50, 1729–1741.
- Mackinnon, M.J. & Read, A.F. (1999). Selection for high and low virulence in the malaria parasite *Plasmodium chabaudi*. *Proc. R Soc. Lond. Ser. B Biol. Sci.*, 266, 741–748.
- Messenger, S.L., Molineux, I.J. & Bull, J.J. (1999). Virulence evolution in a virus obeys a trade-off. *Proc. R Soc. Lond. Ser. B Biol. Sci.*, 266, 397–404.
- Metz, J.A.J., Nisbet, R.M. & Geritz, S.A.H. (1992). How should we define 'fitness' for general ecological scenarios? *Trends Ecol. Evol.*, 7, 198–202.
- Moya, A., Elena, S.F., Bracho, A., Miralles, R. & Barrio, E. (2000). The evolution of RNA viruses: a population genetics view. *Proc. Natl Acad. Sci. USA*, 97, 6967–6973.

- Nowak, M. (1991). The evolution of viruses – competition between horizontal and vertical transmission of mobile genes. *J. Theor. Biol.*, 150, 339–347.
- Nowak, M.A. (1992). What is a quasispecies? *Trends Ecol. Evol.*, 7, 118–121.
- Otto S.P. & Day T. (2007). *A Biologist's Guide to Mathematical Modeling in Ecology and Evolution*. Princeton University Press, Princeton, NJ, USA.
- Pease, C.M., Lande, R. & Bull, J.J. (1989). A model of population-growth, dispersal and evolution in a changing environment. *Ecology*, 70, 1657–1664.
- Price, G. (1970). Selection and covariance. *Nature*, 227, 520–521.
- Price, G.R. (1972). Fisher's 'fundamental theorem' made clear. *Ann. Hum. Genet. Lond.*, 36, 129–140.
- Price, G.R. (1995). The nature of selection. *J. Theor. Biol.*, 175, 389–396.
- Reed, J. & Stenseth, N.C. (1984). On evolutionarily stable strategies. *J. Theor. Biol.*, 108, 491–508.
- Roughgarden, J. (1971). Density-dependent natural selection. *Ecology*, 52, 453–468.
- Sasaki, A. (1994). Evolution in antigen drift/switching: continuously evading pathogens. *J. Theor. Biol.*, 168, 291–308.
- Sasaki, A. & Iwasa, Y. (1991). Optimal growth schedule of pathogens within a host: switching between lytic and latent cycles. *Theor. Popul. Biol.*, 39, 201–239.
- Slatkin, M. (1980). Ecological character displacement. *Ecology*, 61, 163–178.
- Taper, M.L. & Case, T.J. (1992). Models of character displacement and the theoretical robustness of taxon cycles. *Evolution*, 46, 317–333.
- Vincent, T.L., Cohen, Y. & Brown, J.S. (1993). Evolution via strategy dynamics. *Theor. Popul. Biol.*, 44, 149–176.
- Waxman, D. & Gavrillets, S. (2005). 20 questions on adaptive dynamics. *J. Evol. Biol.*, 18, 1139–1154.
- Wilke, C.O. (2003). Probability of fixation of an advantageous mutant in a viral quasispecies. *Genetics*, 163, 467–474.
- Wilke, C.O. (2005). Quasispecies theory in the context of population genetics. *BMC Evol. Biol.*, 5, 44.
- Williams, P.D. & Day, T. (2001). Interactions between sources of mortality and the evolution of parasite virulence. *Proc. R. Soc. Lond. Ser. B Biol. Sci.*, 268, 2331–2337.

## APPENDIX

### SIMULATION DETAILS

Here we describe details of the simulations used to conduct 'experiments' on virulence evolution. Our goal was to simulate a simple digital organism with which we could conduct experiments, rather than to mimic any particular continuous-time model. Consequently, the simulation described here is not meant to be an approximation of any particular model of the text. This is intentional, since the hope is that the analytical results of the text provide important insight and intuition that carry over to real situations that are not necessarily accurately described by these simple models.

We treat each individual in the simulation explicitly (i.e. it is an individual-based simulation) and events occur stochastically with each individual. The basic simulation for each example follows the epidemiological dynamics of two different pathogen strains in discrete time. The 'control' simulations were designed as follows. The population was initialized with  $S(0)$  susceptible individuals, and  $I_1(0)$  and  $I_2(0)$  individuals infected with each strain.

Each generation proceeds as follows: (i) Each individual gives birth to a single offspring with probability  $\exp[-\zeta_j N]$ , where  $j \in \{1, 2\}$ ,  $\zeta_j$  is a positive 'birth rate' parameter, and  $N$  is the total number of individuals in the population at the beginning of the generation (excluding the focal individual). This form of density-dependent reproduction was used as a simple means of regulating population size. All offspring of susceptible hosts are susceptible, but infected hosts produce infected offspring with probability  $f$ . None of the offspring undergo any further events during the generation in which they were produced. For the adults present at the beginning of the generation, the following additional events occur: (ii) Each susceptible host dies (with probability  $d$ ), contacts a randomly chosen individual (with probability  $\xi N$ ), or does nothing. Here,  $\xi$  is a positive parameter restricted to  $\xi \leq 1/N^*$ , where  $N^*$  is the maximum possible population size. Each of these contacts occurs with an infected individual of type  $i$  with probability  $I_i/N$ . Susceptible hosts that contact an infected host of type  $i$  then become infected with probability  $\tau_i$ . Newly generated infectious undergo no further events during the generation in which they were produced. (iii) For each infected host that was present at the beginning of the generation, mortality/recovery/mutation occurs. In particular, each infected host dies from infection (with probability  $\alpha_i$ ), dies from natural causes (with probability  $d$ ), recovers from infection (with probability  $c_i$ ), or 'mutates' to an infected individual carrying the other pathogen type (with probability  $\kappa$ ). We assume  $d + \alpha_i + c_i + \kappa \leq 1$ .

In all simulation results the following parameter values are used:  $\zeta_s = 0.001$ ,  $\zeta_1 = 0.001$ ,  $\zeta_2 = 0.001$ ,  $d = 0.01$ , and  $\xi = 0.00006$ . Note that the parameter for virulence,  $\alpha$ , is analogous to the parameter  $v$  in the analytical models of the text but it is not exactly equivalent.  $\alpha$  is a probability of death from infection whereas  $v$  is a rate of death from infection. Similarly, the product  $\xi\tau$  is analogous to the transmission rate parameter  $\beta$  in the analytical results.

### Experiment 1: effect of background mortality

In these experiments we set  $f = 0$ . The treatment simulations for this experiment were identical to the control simulations except as follows. In the non-replacement protocol, every  $\gamma$  generations, we removed each individual in the population with probability  $\rho$ . In the parallel-

replacement protocol we maintained a parallel simulation identical to the control simulation, and every time we removed  $x$  individuals from the treatment simulation, we replaced them with  $x$  randomly chosen individuals from this parallel stock. In the  $S$ -replacement protocol, every time we removed  $x$  individuals from the treatment simulation, we replaced them with  $x$  susceptible individuals.

### Experiment 2: effect of transmission mode

The treatment simulations for this experiment were identical to the control simulation except as follows. In the VT-removal protocol, every  $\gamma$  generations, we removed all infections that were generated by vertical transmission in that generation, and replaced these with new, horizontally

derived infections. Strain type for these replacements were chosen randomly, in proportion to the frequency of their occurrence at the beginning of that generation. In the HT-removal protocol, every  $\gamma$  generations, we removed all infections that were generated by horizontal transmission in that generation, and replaced these with new, vertically derived infections, and choosing strain type randomly, in proportion to the frequency of their occurrence at the beginning of the generation.

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