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The evolution of resistance against good and bad infections

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Abstract

Opportunities for genetic exchange are abundant between bacteria and foreign genetic elements (FGEs) such as conjugative plasmids, transposable elements and bacteriophages. The genetic novelty that may arise from these forms of genetic exchange is potentially beneficial to bacterial hosts, but there are also potential costs, which may be considerable in the case of phage infection. Some bacterial resistance mechanisms target both beneficial and deleterious forms of genetic exchange. Using a general epidemiological model, we explored under which conditions such resistance mechanisms may evolve. We considered a population of hosts that may be infected by FGEs that either confer a benefit or are deleterious to host fitness, and we analysed the epidemiological and evolutionary outcomes of resistance evolving under different cost/benefit scenarios. We show that the degree of co-infection between these two types of infection is particularly important in determining the evolutionarily stable level of host resistance. We explore these results using the example of CRISPR-Cas, a form of bacterial immunity that targets a variety of FGEs, and we show the potential role of bacteriophage infection in selecting for resistance mechanisms that in turn limit the acquisition of plasmid-borne antibiotic resistance. Finally, beyond microbes, we discuss how endosymbiotic associations may have shaped the evolution of host immune responses to pathogens.

Introduction

The microbial world is enormously promiscuous, with abundant opportunities for genetic exchange via horizontal gene transfer (HGT) (Koonin *et al.*, 2001; Redfield, 2001; Gogarten, 2009; Zhaxybayeva & Doolittle, 2011). For example, it is estimated that over 80% of all prokaryote genes were horizontally transferred at some point during their evolutionary history (Dagan *et al.*, 2008). HGT may occur through several routes, including the uptake of free DNA in the environment (Lorenz & Wackernagel, 1994), the exchange of conjugative plasmids (Norman *et al.*, 2009), or during infection by bacteriophages or transposable elements capable of integrating into the host genome (Chen & Novick, 2009). There may be substantial benefits associated with genetic exchange with many of these foreign genetic elements (FGEs). Conjugative plasmids are a known source of antibiotic resistance genes and virulence factors (Chen & Novick, 2009; Norman *et al.*, 2009) and may also promote biofilm formation (Ghigo, 2001). HGT therefore offers a powerful means of colonizing new ecological niches through the acquisition of novel functions (Ochman *et al.*, 2000; Koonin *et al.*, 2001; Zhaxybayeva & Doolittle, 2011).

Genetic exchange may also have negative consequences for host fitness (Vos, 2009). Plasmids and transposable elements are often costly when they are first acquired (Andersson & Levin, 1999) and this may hamper the long-term maintenance of these elements in bacterial populations (Stewart & Levin, 1977; Lundquist & Levin, 1986; Bergstrom *et al.*, 2000). Although these fitness costs may be reduced via compensatory mutations (Dahlberg & Chao, 2003; Dionisio *et al.*, 2005), some FGEs may integrate into the host genome,

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Correction added on 21st January 2014, after first online publication: missing grey shaded area in Fig. 2a added.

causing a 'recombination load' due to the disruption of functional genes (Otto & Lenormand, 2002; Agrawal, 2006). Costs may be even more severe: some bacteriophages use the plasmid conjugation apparatus as the mode of entry into bacterial cells, making harbouring a plasmid potentially deadly (Caro & Schnös, 1966; Jalasvuori *et al.*, 2011). Given these potential costs, several mechanisms have evolved that limit the acquisition and spread of FGEs. These include DNA restriction mechanisms against plasmids (Thomas & Nielsen, 2005) and restriction modification and abortive infection mechanisms against phage infection (Labrie *et al.*, 2010). Other mechanisms suggest a shared evolutionary history between several FGEs. For example, some plasmids have evolved to conditionally repress sex-pili formation, despite reducing their rate of transmission by several orders of magnitude (Koraimann *et al.*, 1991), presumably because this reduces the spread of phages that infect via the conjugation apparatus (Anderson, 1968; Jalasvuori *et al.*, 2011).

Continued infection by both phage and plasmids is also apparent in CRISPR-Cas, a sequence-specific form of bacterial immunity that targets homologous DNA of foreign origin (Horvath & Barrangou, 2010; Jiang *et al.*, 2013). CRISPR-Cas has been shown to target a variety of FGEs, including phage (Barrangou *et al.*, 2007; Garneau *et al.*, 2010), plasmids (Marraffini & Sontheimer, 2008; Garneau *et al.*, 2010), and has also been found to limit natural transformation of foreign DNA in competent bacterial cells (Bikard *et al.*, 2012; Jorth & Whiteley, 2012). Resistance mechanisms such as CRISPR-Cas therefore present a potential dilemma: they may protect cells from the negative consequences of infection, but also prevent infection by FGEs conferring potential benefits (Levin, 2010). The fitness advantage of such a resistance mechanism is therefore variable, making its evolution hard to predict in the wild, where the frequency of infection by different types of FGEs is highly variable.

Here, we ask the general question of how such resistance mechanisms evolve given continued mixed infection by FGEs conferring opposing and often variable costs and benefits. We study these conditions by considering a general epidemiological model that captures the infection dynamics of many different foreign genetic elements. We explore several infection scenarios, starting with infection by a single infectious element and then expand to more complex scenarios where co-infection by both beneficial and deleterious forms of HGT is possible. We ask how resistance evolves as a function of the cost of carrying this resistance mechanism, and the rates of infection by good or bad genes.

A simple model of infections by foreign genetic elements

We develop and analyse a simple yet general epidemiological model that captures the dynamics of infection by foreign genetic elements carrying either genes with beneficial effects (which we call FGE_{Good}) or with deleterious effects (which we call FGE_{Bad}), and we study the epidemiological and evolutionary outcomes of resistance against them.

Epidemiological dynamics

We consider a population of susceptible hosts, with density S, reproducing at a rate b and dying at a rate d. Fecundity is assumed to be density-dependent and κ measures the intensity of density dependence. In a first step of the analysis, we do not explicitly model the epidemiological dynamics of the infectious elements, which allows the analysis to be considerably simplified. Instead, we assume that there is a constant rate of infection (the force of infection ψ), but this assumption is relaxed later on in the analysis. Because we consider two types of infectious particles, we define ψ_G and ψ_B as the force of infection by FGE_{Good} and FGE_{Bad}, respectively. Susceptible hosts may thus yield hosts infected by good or bad FGEs with densities I_G and I_B, respectively (Fig. 1). We allow co-infections to occur but this event is controlled by the parameter σ that measures the susceptibility to co-infections (i.e. when $\sigma = 0$ no co-infections are allowed). Infections may alter various properties of the hosts and in particular their fecundity and/or their survival rates which allows for considering a broad range of scenarios for the costs and benefits of the infections. Infected hosts may clear the infection with rates γ_G and γ_B, depending on the type of infection (Fig. 1). For the sake of simplicity, we assume that clearance does not lead to acquired immunity, but to full susceptibility. This is equivalent to assuming a great diversity of infectious types such that even if one type is cleared, the host is always susceptible to another type, which occurs, for example, in the case of CRISPR-Cas-mediated defence. In addition, we assume that when the infected host reproduces it always transmits the FGE vertically (i.e. 100% fidelity of vertical transmission).

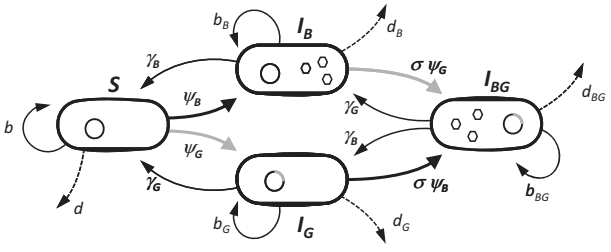

Fig. 1 Schematic representation of the full life cycle with uninfected cells (with density S), cells infected with the FGE_{Bad} (I_B), cells infected with the FGE_{Good} (I_G) and cells co-infected (I_{BG}). See the main text and eqn 1 for the definition of all the parameters of this model.

There are many different ways in which the host can defend itself against an infection, and in the following, we consider that host resistance, π, acts through a reduction in the force of infection. The above life cycle yields the following set of differential equations (see also Fig. 1):

$$\begin{aligned}
\dot{S} &= b(1-\kappa N)S+\gamma_B I_B+\gamma_G I_G-(d+(1-\pi)(\psi_B+\psi_G))S\\
\dot{I}_G &= b_G(1-\kappa N)I_G+(1-\pi)\psi_G S\\
&\quad -(d_G+\gamma_G+\sigma(1-\pi)\psi_B)I_G+\gamma_B I_{BG}\\
\dot{I}_B &= b_B(1-\kappa N)I_B\\
&\quad +(1-\pi)\psi_B S-(d_B+\gamma_B+\sigma(1-\pi)\psi_G)I_B+\gamma_G I_{BG}\\
\dot{I}_{BG} &= b_{BG}(1-\kappa N)I_{BG}+\sigma(1-\pi)(\psi_B I_G+\psi_G I_B)\\
&\quad -(d_{BG}+\gamma_B+\gamma_G)I_{BG}
\end{aligned} \tag{1}$$

where $N = S + I_B + I_G + I_{BG}$ is the total density of hosts.

As discussed below, the above dynamical system relies on several simplifying assumptions. But first, our aim is to develop a minimal model that can be used to explore various evolutionary scenarios.

Evolutionary dynamics

To explore the evolutionary dynamics of resistance in this model we assume, as in classical models of host resistance evolution, that resistance may be costly (Boots & Haraguchi, 1999). More specifically, we assume that higher levels of resistance may reduce the fecundity of the hosts in the following way:

$$b(\pi) = b_{\max} e^{-c\pi} \tag{2}$$

where $b_{\max}$ is the maximal fecundity of the host (i.e. the fecundity of a fully susceptible host) and c is the cost of resistance. Although the evolutionary outcome depends on specificities of the cost functions, the evolutionary analysis does not (Appendix S1). Similarly, the fecundity of the other types of hosts is $b_i(\pi)=F_i b(\pi)$ where $i = B$, G or BG, and F_i is the coefficient measuring the effect of the infection on fecundity.

Under the assumption that the mutation rate is sufficiently low, the evolutionary dynamics is much slower than the ecological dynamics, which allows decoupling the ecological and the evolutionary dynamics. First, the system reaches an endemic equilibrium where three (or four if co-infections are allowed) types of host coexist (Fig. 1). Second, to analyse the evolution of host resistance, we study the fate of a rare host variant that appears by mutation. The fixation of the new mutation requires that the per-generation invasion fitness of the mutant $R_m > 1$ (Appendix S1). Three case studies are considered below.

Case study 1: resistance against FGE_{Bad}

Let us first consider the simple situation where the host can be infected by a single FGE_{Bad}. The above invasion criterion is reduced down to the classical results of resistance evolution when only a single FGE is allowed to circulate in the host population Boots *et al.* (2009). When the FGE is extremely deleterious (no reproduction and no recovery is possible after infection), the per-generation invasion fitness of the mutant yields (see Appendix S1 for a more general scenario):

$$R_m(\pi) = \frac{b(\pi)}{d+(1-\pi)\psi_B} \tag{3}$$

The invasion fitness does not depend on the survival of infected hosts because castration by the parasite prevents them from contributing to the evolution of the host population. In this case and under the specific assumptions used to model the cost of resistance (eqn 2), only two outcomes are evolutionarily stable: either no resistance or full resistance (Fig. 2, left panel). The endpoint may depend on the initial level of resistance. The threshold value below which the host evolves towards no resistance is:

$$\pi_c = 1-\frac{1}{c}+\frac{d}{\psi_B} \tag{4}$$

In particular, the host evolves full resistance (i.e. $\pi = 1$) whatever the initial value of resistance π when $c<\frac{\psi_B}{d}$. In other words, full resistance is expected to evolve when the cost of resistance is sufficiently low relative to the force of infection (Fig. 2a). This all-or-nothing evolutionary response contrasts with the evolution of an intermediate level of resistance in some models (Boots *et al.*, 2009). This is due to the lack of epidemiological feedback, as in the present model, the increase in the level of resistance does not reduce the force of infection.

The present model can be readily modified to incorporate such a feedback if one assumes that the force of infection is tied to the prevalence of the disease. For example, one can assume that each infected host produces free particles of the bad FGE at a constant horizontal transmission rate β_B and the force of infection becomes: $\psi_B = \beta_B I_B$ (Appendix S1). In this case, the maintenance of the FGE_{Bad} is a necessary condition for the evolution of resistance. As expected, intermediate levels of resistance can be selected for because selection towards very a high level of resistance is hampered by reductions in the prevalence of the pathogen (Fig. 2b). Note that evolutionary bistability can still be observed for intermediate levels of the cost of resistance.

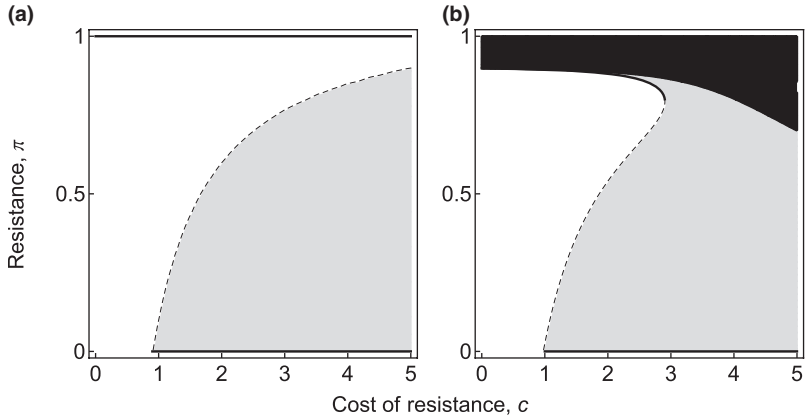

Fig. 2 Evolutionary dynamics of host resistance, π, against a single pathogen (i.e. FGE_{Bad}) for increasing levels of the cost of resistance. The white and grey areas indicate when selection for host resistance is positive or negative, respectively. The bold line indicates the location of evolutionary stable equilibria. The dashed line indicates unstable evolutionary equilibria. In (a), we consider the situation where the force of infection is fixed: $\psi_B = 10$. In (b), the epidemiology is allowed to feed back on the force of infection: $\psi_B = \beta_B I_B$ with $\beta_B = 5$. In this scenario, the pathogen may go extinct when the level of resistance becomes very high (black area). Other parameters values: $d = 1$, $d_B = 50$, $\gamma_B = 0$, $F_B = 0$, $b_{max} = 50$, $\kappa = 0.01$.

Case study 2: resistance against bad and good FGEs

Let us now consider situations where the host can be infected by FGE_{Good}. As expected, when the only FGE has beneficial effects on the host, resistance is always selected against [see also Fig. 6D in (Levin, 2010)]. However, if both good and bad FGEs are simultaneously present, the evolution of resistance depends on the balance between the opposite selective pressures of FGE_{Good} and FGE_{Bad}.

Figure 3 shows the effect of increasing the force of infection of FGE_{Good} (assumed to increase the fecundity of the host) on the evolution of resistance. In the absence of co-infection ($\sigma = 0$) with the FGE_{Bad} (assumed to prevent reproduction and recovery and to increase mortality), increasing the risk of infection by FGE_{Good} selects for lower resistance levels, as expected. Interestingly, there is a threshold value of the force of infection by FGE_{Good} above which host resistance is selected against in the absence of a direct cost of resistance (see A15 in Appendix S1). When there is some

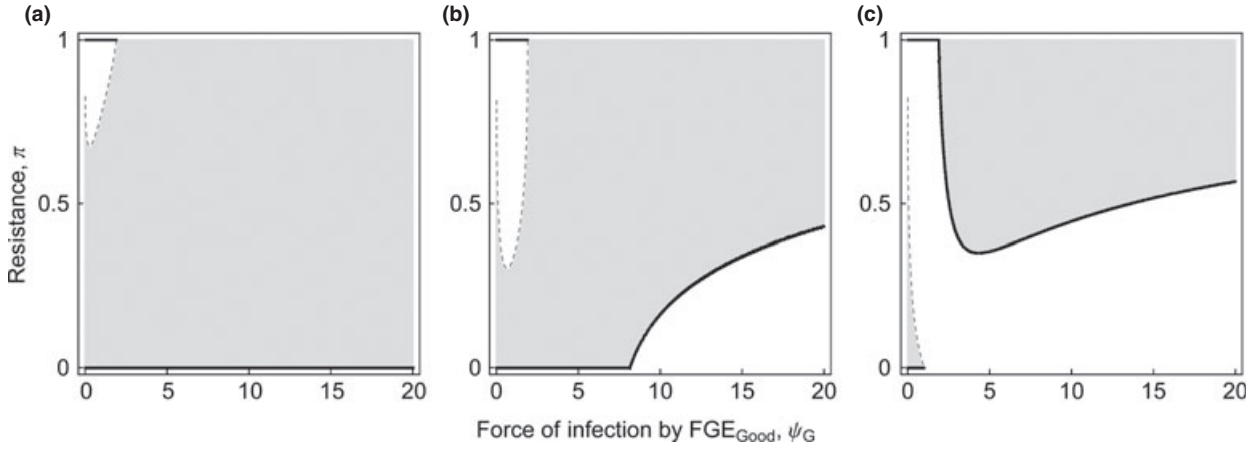

Fig. 3 Evolutionary dynamics of host resistance, π, against increasing force of infection of FGE_{Good}, ψ_G, for various levels of co-infection: $\sigma = 0$,0.075, 0.085 in a, b and c, respectively. As in Fig. 2, the white and grey areas indicate when selection for host resistance is positive or negative, respectively. The bold line indicates the location of evolutionary stable equilibria. The dashed line indicates unstable evolutionary equilibria. Other parameters values: $\psi_B = 2$, $d = d_G = 1$, $F_G = 1.5$, $\gamma_G = 1$, $d_B = d_{BG} = 6$, $F_B = F_{BG} = 0$, $\gamma_B = 0$, c = 0.1, $b_{max} = 10$.

risk of co-infection $\sigma > 0$), some intermediate level of resistance can become evolutionarily stable (Fig. 3). This arises because as the force of infection ψ_G increases it becomes important to protect hosts infected with the FGE_{Good} against FGE_{Bad} (selection for resistance). However, total resistance is no longer an evolutionarily stable strategy because it prevents the host from acquiring a beneficial FGE_{Good} in the first place. So when co-infection is possible, the evolutionary outcome may be (Fig. 3b): (i) full resistance when the force of infection by FGE_{Good} ψ_G is low, (ii) no resistance when ψ_G is intermediate (low prevalence of co-infection) and (iii) intermediate resistance when ψ_G is high (high prevalence of co-infection).

We now consider the evolution of resistance under the opposite scenario, where there is an increase in the force of infection by FGE_{Bad} for a fixed force of infection by FGE_{Good}. Fig. 4 shows, as expected, increasing the risk of infection by parasites tends to select for higher levels of resistance. Again, low levels of co-infections yield an all-or-nothing evolutionary outcome. Some co-infection can select for intermediate levels of resistance that balance the cost and the benefit of being resistant to foreign genetic elements (Fig. 4).

As in the previous subsection, it is possible to incorporate an epidemiological feedback on the evolution of resistance when both FGE_{Bad} and FGE_{Good} are circulating. For instance, one can assume that hosts infected with bad or good FGEs produce free particles at rates β_B and β_G, respectively. The analysis of this model is more complicated because one-first needs to characterize the conditions for the coexistence of the host with both FGE_{Bad} and FGE_{Good}. We focused on the evolution of host resistance for increasing rates of infections by bad or good FGEs, and we confirmed the qualitative results obtained in the absence of epidemiological feedbacks (compare Fig. 3 with Fig. S1 and Fig. 4 with Fig. S2). Regardless of the occurrence of an epidemiological feedback, it is clear that allowing for co-infection between FGEs has a huge effect on the evolution of resistance.

Case study 3: antibiotic use and selection for resistance against plasmids

In a final case study, we consider a situation where the effect of one of the foreign genetic element varies in time. For example, consider a scenario where bacteria can be infected by a lytic phage (the FGE_{Bad}) or by a plasmid that carries an antibiotic resistance gene. In the absence of antibiotics in the environment, carrying the plasmid is costly for the bacteria (reduced fecundity). When the antibiotic is present, however, cells that do not carry the plasmid have a lower survival rate. As a consequence, the system switches from a situation with two FGE_{Bad} to a situation with one FGE_{Bad} (the phage) and one FGE_{Good} (the plasmid).

Figure 5 shows the evolutionary dynamics of host resistance to infection by FGEs. As expected, before the use of antibiotics, the plasmid induces a fecundity cost and so the host evolves towards high levels of resistance to infection. After introducing antibiotics, the level of antibiotic resistance increases (because plasmids now offer a benefit) and the host evolves towards lower levels of resistance to infection. However, low levels of host resistance against plasmid infection can be reverted, even in the presence of antibiotics, if the force of infection of FGE_{Bad} increases (Fig. 5). This is the case especially when co-infection is possible, where bacteria carrying a plasmid may also acquire an infection by a

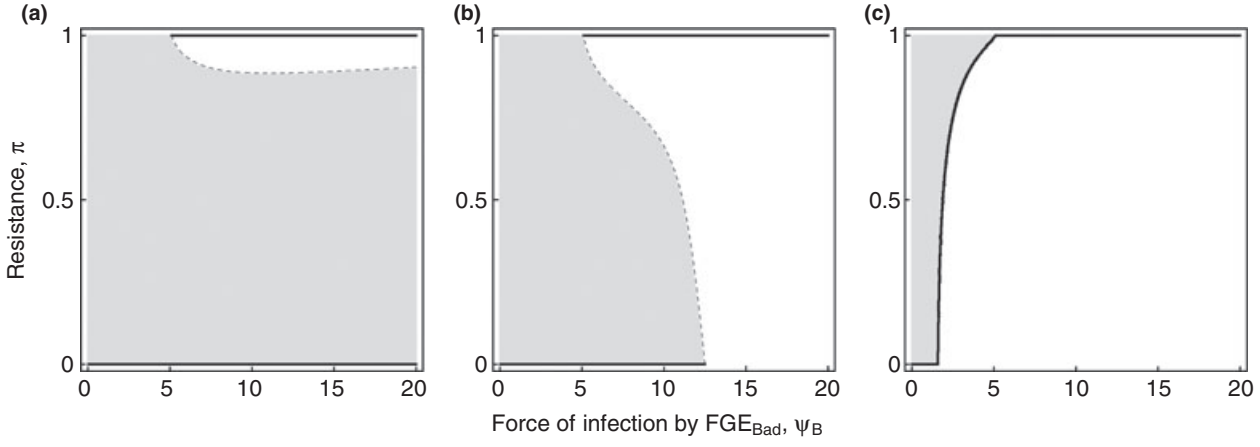

Fig. 4 Evolutionary dynamics of host resistance, π, against increasing force of infection of FGE_{Bad}, ψ_B, for various levels of co-infection: $\sigma = 0$, 0.015, 0.1 in a, b and c, respectively. As in Fig. 2, the white and grey areas indicate when selection for host resistance is positive or negative, respectively. The bold line indicates the location of evolutionary stable equilibria. The dashed line indicates unstable evolutionary equilibria. Other parameters values: $\psi_G = 5$, $d = d_G = 1$, $F_G = 1.5$, $\gamma_G = 1$, $d_B = d_{BG} = 6$, $F_B = F_{BG} = 0$, $\gamma_B = 0$, c = 0.1, $b_{max} = 10$.

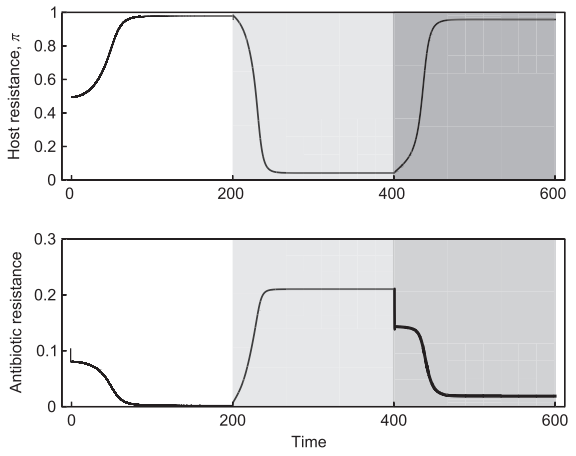

Fig. 5 Effect of antibiotics and phages on the evolution of host resistance, π, and on the evolution of antibiotic resistance (see case study 3 in the main text). We performed deterministic numerical simulations assuming that host resistance is a quantitative trait varying between 0 and 1 discretized in 20 genotypes (code available upon request). Stepping-stone mutation occurs upon host reproduction with a probability $\mu = 0.05$. Antibiotic resistance is assumed to be carried by a plasmid (FGE_{Good}). In the first 200 time steps, there is no antibiotic in the environment. From $t = 200$ until the end of the simulation, an antibiotic is introduced and this increases the mortality of all the bacteria that do not carry the plasmid with the antibiotic resistance (grey shading). As a consequence, antibiotic resistance becomes advantageous, and host resistance is counter-selected. From $t = 400$ until the end of the simulation, the force of infection by lytic phages is increased (dark grey shading). This selects for higher level of host resistance and, as a consequence, for lower level of antibiotic resistance. For the sake of simplicity, we do not consider the feedback of epidemiology on the forces of infection in this scenario. For $t < 100$: $\psi_G = 1$, $\psi_B = 1.6$, $\sigma = 1$, $F_G = 0.8$, $F_B = F_{BG} = 0$, $d = 1$, $d_G = 1$, $d_B = d_{BG} = 3$, $\gamma_G = 4$, $\gamma_B = 0$, c = 0.5, $b_{max} = 10$, $\kappa = 1$. For $t > 200$: $d = 4$, $d_B = 6$. For $t > 400$: $\psi_B = 4$.

lytic phage. This can select for higher levels of resistance against infection and, as a consequence, limit the acquisition of the plasmid and lower the equilibrium level of antibiotic resistance (Escobar-Páramo *et al.*, 2012; Zhang & Buckling, 2012). Note, however, that this result depends on the whole life cycle of both the bad and the good FGEs. In particular, selection against antibiotic resistance is lower when the clearance rate of the good FGE is low (Fig. S3) because resistance against infection does not limit the spread of the plasmid, which spreads both horizontally and vertically.

Discussion

The main aim of this study was to gain greater understanding into the evolution of host resistance, recognizing that infectious elements may cause a range of effects on host fitness from potentially beneficial to highly deleterious (Michalakis *et al.*, 1992; Casadevall & Pirofski, 2003; Fellous & Salvaudon, 2009; Vos, 2009; Schneider, 2011). Our results highlight that the outcome of resistance evolution depends not only on the forces of infection of beneficial and deleterious foreign genetic elements (FGEs), but also critically on whether co-infection is possible.

Co-infection matters: taking the bad with the good

Our analysis confirmed that full resistance is expected to evolve under a broad range of conditions when the FGE is deleterious, but that resistance is always counter-selected when the FGE has beneficial effects on the host. When both types are present (but when co-infection does not occur), we found that increasing the risk of infection by FGE_{Good} selects for lower resistance levels, because of the increasing benefit of acquiring a FGE_{Good}. The benefit of FGE_{Good} is two-fold. First, it carries some intrinsic benefit to the host. Second, when co-infection is not allowed, it protects the host against the infection by the FGE_{Bad}. However, when co-infection is possible, it becomes more important to protect the host against the deleterious FGEs, but compared with the scenario where only deleterious FGEs are present, total resistance is no longer the evolutionarily stable strategy and intermediate resistance evolves (Figs 2 and 3). We interpret this result in light of the potential benefits to host fitness under a scenario of co-infection: whereas full resistance protects from potential deleterious infections, it also prevents the host from acquiring an FGE_{Good} in the first place. This result highlights that resistance evolution is highly contingent on a host's relative exposure to the many types of infectious genetic elements present in the environment and that the likelihood of co-infection is a powerful determinant of the evolutionarily stable level of resistance.

CRISPR: microbial defence for better or worse

One of the motivations behind the present study was the observation that CRISPR-Cas, a sequence-specific form of immunity in microbes, limits horizontal gene transfer and clears infection by viruses, plasmids and transposable elements (Mojica *et al.*, 2005; Barrangou *et al.*, 2007; Marraffini & Sontheimer, 2008; Garneau *et al.*, 2010; Bikard *et al.*, 2012; Jorth & Whiteley, 2012). CRISPR-Cas therefore offers the perfect example of a resistance mechanism that may be constrained in its evolution by the variable costs and benefits of infection.

CRISPR-Cas offers a potentially powerful tool to study co-evolutionary dynamics between microbial hosts and the FGEs that infect them (Vale & Little, 2010). Recent theoretical work has attempted to capture the complex mechanistic detail of CRISPR-Cas, testing the necessary conditions for its maintenance in microbial populations (Levin, 2010), or its role in pro-

moting microbial diversity (Childs *et al.*, 2012). Our goal in the present study was not to describe the intricacies of this defence system, but to provide a simple and tractable theoretical framework to allow resistance evolution to be followed under both beneficial and deleterious forms of infection. However, our model still captures the general properties of CRISPR: 1) it is a resistance mechanism that may prevent infection by foreign genetic elements having either beneficial or deleterious effects on the host (Garneau *et al.*, 2010; Bikard *et al.*, 2012) and 2) resisting infection restores host cells to a state where they remain susceptible to infection from a large pool of FGEs still present in the environment (Andersson & Banfield, 2008; Held *et al.*, 2010).

The evolution of this microbial 'immune system' (Horvath & Barrangou, 2010) has certainly been influenced by an often volatile history of infection by both beneficial and deleterious forms of infection. For example, the occurrence of nonfunctional CRISPR-Cas in *E.coli* (Mojica & Díez-Villaseñor, 2010; Pul *et al.*, 2010; Westra *et al.*, 2010) would appear counter-intuitive given its widespread role in antiviral defence. However, this might be expected if the FGEs experienced by *E. coli* over evolutionary time were more likely to be beneficial than deleterious. Recent bioinformatic analyses of sequenced *E. coli* strains found a predominance of spacers originating from plasmids known to carry antibiotic resistance, rather than bacteriophages (Touchon & Rocha, 2010). Given the potential benefits of plasmids (antibiotic resistance, virulence factors), the history of infection in *E. coli* as revealed by its CRISPR-Cas content would suggest a scenario where the force of infection of beneficial FGEs was higher than that of deleterious FGEs, which should select against resistance. This may be one possible explanation for CRISPR repression in *E. coli*.

Plasmid-borne antibiotic resistance may also have shaped the evolution of CRISPR-Cas in other microbial species. Palmer and Gilmore (Palmer & Gilmore, 2010) noted that acquisition of multidrug resistance over time in strains of *Enteroccocus faecalis* also correlated strongly with the loss CRISPR-Cas machinery during the same time period (Palmer & Gilmore, 2010) (but see Touchon *et al.*, 2012). Presumably, the benefit of being able to acquire plasmids carrying antibiotic resistance meant that mutant strains lacking CRIPSR had an advantage and could therefore increase in frequency. This observation raises the intriguing possibility that antibiotic use could indirectly select against mechanisms that protect hosts from horizontal gene transfer, when its effects are predominantly beneficial for the host (in this case, acquiring resistance to drugs). Further experimental evidence is provided by a recent study on *Staphylococcus epidermis* showing that when confronted with a good FGE (an antibiotic resistant plasmid), the host readily evolves the ability to shut down immunity (deletion of the spacer targeting the good FGE, or deletion of the whole CRISPR locus) to acquire this new function (Jiang *et al.*, 2013). Hence, the lability of CRISPR immunity may be an adaptation to the frequent encounter with good FGE. We explored this scenario in case study 3 and found concurring results. Initially costly plasmids select for increased levels of resistance to infection by this FGE, but introducing antibiotics switches plasmids from costly to beneficial, selecting instead for reduced resistance to infection and an increase in antibiotic resistance (Fig. 4). This result underlines the importance of the context-dependent nature of infection when considering the evolution of resistance to infection.

The enemy of my enemy is my friend: using phage to reduce antibiotic resistance

In the same case study 3, we further explored what effect the addition of deleterious FGEs (e.g. bacteriophage) might have on a defence system that targets both good and bad infection. Given our initial results showing that increasing the force of infection of bad FGEs generally selected for high levels of resistance to infection, we enquired whether this would also be the case in the presence of a beneficial FGE. Our simulation results showed that when co-infection between good and bad FGEs occurs, increasing the force of infection of bad genes can indeed select for increased resistance to infection (Fig. 4). This result may have implications for managing antibiotic resistance. Using the same example of phage and antibiotic resistance conferring plasmids, this result implies that even in the presence of antibiotics, it is possible to select against plasmid-borne antibiotic resistance by selecting for increased resistance against phage (and as a consequence, against plasmids too). A scenario similar to this one was recently addressed experimentally using *E.coli*, plasmids carrying drug resistance genes and the plasmid-dependent lytic phage PRD1 (Jalasvuori *et al.*, 2011). As in our model, in this system, *E.coli* hosts can be infected by a FGE_{Good} in the form of a plasmid that confers antibiotic resistance, and further co-infected by a FGE_{Bad} because the bacteriophage in question uses the plasmid conjugation apparatus as its mode of entry into the host cell. This work showed that adding phage can select against plasmid-carrying host cells and that therefore bacteriophages could in principle play an important role in limiting the spread of antibiotic resistance (Escobar-Páramo *et al.*, 2012; Refardt, 2012; Zhang & Buckling, 2012).

Good and bad infection in insects and beyond : has symbiosis shaped immune responses ?

Beyond the microbial world, the response against infection in other taxa may have also been shaped by

co-infection by beneficial and deleterious forms of infection. For example, the pea aphid *Acyrthosiphon pisum* is an example of a species that must frequently deal with a variety of viral, bacterial and fungal pathogens (Hagen & VanDenBosch, 1968). In addition, pea aphids are rather unique in that they also enjoy intimate liaisons with a number of gram-negative bacterial symbionts. These symbionts may be obligate such as *Buchnera aphidicola* or facultative like *Hamiltonella defensa* and may synthesize many essential aminoacids, play a role in heat tolerance and promote resistance to parasites (Montllor *et al.*, 2002; Oliver *et al.*, 2005; Russell & Moran, 2006). Given this variable infectious context, we may wonder whether the aphid innate immune response has been shaped by an evolutionary history of exposure to both deleterious pathogens and beneficial symbionts. Using a combination of bioinformatics and functional assays, Gerardo and colleagues (Gerardo *et al.*, 2010) recently characterized the aphid immune response and found that it lacks several of the genes thought to be central to arthropod innate immunity. These include peptidoglycan receptor proteins (PGRPs) and most of the IMD pathway, which are critical for the recognition, signalling and response to infection by gram-negative bacteria (Steiner, 2004; Kaneko & Silverman, 2005). Given that these immune genes are conserved in other insects such as flies, mosquitos, bees and beetles, Gerardo and colleagues discussed the possible connection between the absence of this arm of the immune response and the presence of potentially valuable bacterial symbionts. Although certainly anecdotal, there is mounting evidence that co-infection by both deleterious and beneficial forms of bacterial infection may have shaped the immune response in both invertebrates (Elsik, 2010) and vertebrates, including humans (McFall-Ngai, 2007; Lee & Mazmanian, 2010).

Perspectives

In the present study, we focused on a particular scenario where resistance against a pathogen may have a pleiotropic effect on resistance against a good gene. Our work has highlighted how the evolution of host traits, such as resistance to infection, is strongly determined by the great diversity of foreign genetic elements, and especially by their variable costs and benefits to the host when co-infection is possible. Determining how commonly co-infection occurs between the great diversity of FGEs present in nature is therefore a potentially useful line of research, with clear implications for the evolution of microbial resistance. We focused specifically on one type of resistance (π, the ability to reduce the force of infection) but the current model could also be expanded in the future to study the evolution of a variety of host traits against pathogens (and the co-evolution between them), with distinct evolutionary outcomes (Boots & Bowers, 1999; Gandon & Michalakis, 2000; Débarre *et al.*, 2012; Garnier *et al.*, 2012). These may include anti-infection mechanisms that reduce infection (i.e. decrease in the force of infection of FGE_{Bad} ψ_B), anti-growth mechanisms (that increase γ_B, the clearance rate of deleterious infections), or damage control mechanisms that reduce the cost of the infection [i.e. decrease in the death rate d_B (Vale *et al.*, 2011) and/or increase in the birth rate b_B (Vale & Little, 2012)], promoting disease tolerance (Best *et al.*, 2008, 2010; Ayres & Schneider, 2011; Medzhitov *et al.*, 2012). Further, the general framework presented could equally be expanded to model the effects of a great variety of infectious elements, be they beneficial or deleterious, affecting host fecundity or survival. Ultimately, understanding the great diversity of extant host defence strategies against infection will require taking into account the equally great diversity, and context-dependent nature, of infectious elements.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 The evolution of resistance against good and bad infections.

Figure S1 Evolutionary dynamics of host resistance, π, against increasing the horizontal transmission rate of FGE_{Good}, β_G, for two levels of coinfection: $\sigma = 0$ and $\sigma = 0.2$ in A and B, respectively.

Figure S2 Evolutionary dynamics of host resistance, π, against increasing the horizontal transmission rate of FGE_{Bad}, β_B, for two levels of coinfection: $\sigma = 0$ and $\sigma = 0.5$ in A and B, respectively.

Figure S3 Effects of antibiotics and phages on the evolution of antibiotic resistance.

Correction added on 21st January 2014, after first online publication: missing grey shaded area in Fig. 2a added.

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