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The social evolution of bacterial pathogenesis

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Many of the genes responsible for the virulence of bacterial pathogens are carried by mobile genetic elements that can be transferred horizontally between different bacterial lineages. Horizontal transfer of virulence-factor genes has played a profound role in the evolution of bacterial pathogens, but it is poorly understood why these genes are so often mobile. Here, I present a hypothetical selective mechanism maintaining virulence-factor genes on horizontally transmissible genetic elements. For virulence factors that are secreted extracellularly, selection within hosts may favour mutant 'cheater' strains of the pathogen that do not produce the virulence factor themselves but still benefit from factors produced by other members of the pathogen population within a host. Using simple mathematical models, I show that if this occurs then selection for infectious transmission between hosts favours pathogen strains that can reintroduce functional copies of virulence-factor genes into cheaters via horizontal transfer, forcing them to produce the virulence factor. Horizontal gene transfer is thus a novel mechanism for the evolution of cooperation. I discuss predictions of this hypothesis that can be tested empirically and its implications for the evolution of pathogen virulence.

Keywords: host–parasite evolution; virulence; cooperation; horizontal gene transfer; selfish genetic elements; pathogenicity islands

1. INTRODUCTION

Bacterial genomes often contain mobile genetic elements such as conjugative plasmids or lysogenic phages. These elements can be inherited vertically during cell division or can be transmitted horizontally between different bacterial lineages. They can also impose substantial fitness costs on the bacteria that carry them (Lenski & Bouma 1987; Bull *et al.* 1991) and in this sense may be molecular parasites. Sometimes, however, mobile elements carry genes that are beneficial to bacteria, including genes for such ecologically important traits as antibiotic resistance (Falkow 1975), pathogen virulence (Kaper & Hacker 1999), symbiotic nitrogen fixation (Van Rhijn & Vanderleyden 1995) and the production of allelopathic bacteriocins (Riley & Gordon 1999). In addition, mobile elements can serve as vehicles for the horizontal transfer of genes between distantly related bacterial species, contributing to a large part of bacterial adaptation and speciation (Ochman *et al.* 2000; Karaolis *et al.* 1995; Whittam 1998).

Despite this important role for plasmids and phages in the ecology and evolution of bacteria, several crucial questions remain unanswered. What evolutionary mechanisms maintain the mobility of the genes borne by these elements? Why have these genes not been sequestered as normal chromosomal genes without the fitness costs of mobile elements? And why is it that across bacterial species certain types of genes are consistently carried by mobile elements, while other types of genes, such as those for housekeeping functions, are always non-mobile? Underlying these questions is the more fundamental issue of how natural selection, in general, creates cooperative higher-level units of biological organization out of smaller separate ones. Understanding the evolutionary processes maintaining genes on mobile genetic elements may shed light on processes likely to have been operating during the evolutionary transition from primitive cells occupied by many independent replicators to cells with large co-replicating chromosomes of linked genes (Maynard Smith & Szathmáry 1995).

One potential explanation for why certain types of genes are carried by mobile genetic elements is that these genes are only useful in certain environments or at certain times. Sometimes-useful genes linked to horizontally transmissible elements could out-compete non-mobile versions of the same genes by associating with bacterial genotypes of greater fitness or by associating with a variety of different bacterial ecotypes (Bergstrom *et al.* 2000). Horizontal transfer could also allow sometimes-useful genes to colonize bacterial populations already occupying habitats in which these genes are favoured (Eberhard 1990).

These hypotheses, however, have trouble accounting for the mobility of virulence-factor genes, genes whose products are responsible for the morbidity and mortality caused by bacterial pathogens. Several functionally diverse virulence factors are carried by mobile elements in many different pathogen species (table 1). While it can be argued that virulence factors are only sometimes useful (Eberhard 1990), it is hard to reconcile the action of the above mechanisms with the sometimes strong association between specific virulence-factor genes and specific chromosomal lineages. For example, pathogenic virulence-factor-bearing strains of *Vibrio cholerae* are also genetically distinct from naturally occurring non-pathogenic strains at several non-mobile chromosomal loci (Karaolis *et al.* 1995; Beltrán *et al.* 1999). This suggests that the rate of transfer of virulence-factor genes between pathogenic and non-pathogenic strains is quite low and argues against the maintenance of virulence-factor mobility by mechanisms requiring frequent transfer between ecotypically distinct bacterial strains.

In this paper, I present a hypothetical mechanism that can explain the selective maintenance of virulence-factor genes on horizontally transmissible genetic elements

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Table 1. *Examples of bacterial pathogens with horizontally transmissible virulence factors*

pathogen	virulence factor	secreted?	genetic element	reference
Vibrio cholerae	cholera toxin	yes	phage CTXΦ	Waldor & Mekalanos 1996
	toxin coregulated pilus	no	phage VPIΦ	Karaolis *et al.* 1999
Enterotoxigenic *Escherichia coli*	LT and ST toxins	yes	Ent plasmids	Smith & Halls 1968
	CFA pili	no	Ent plasmids	Murray *et al.* 1983
Enterohaemorrhagic *Escherichia coli*	shiga toxins	yes	phages H19B, 933W	O'Brien *et al.* 1984
Salmonella enterica	*spv* gene products	yes	pSLT virulence plasmid	Ahmer *et al.* 1999
	SopE type III effector protein	yes	phage SopEΦ	Mirold *et al.* 1999
	superoxide dismutase SodC	no	phage Gifsy-2	Figueroa-Bossi & Bossi 1999
Corynybacterium diptheriae	diptheria toxin	yes	phage β	Groman 1953
group A streptococci	pyrogenic exotoxin A	yes	phage T12	Johnson & Schlievert 1984; Weeks & Ferretti 1984
Staphylococcus aureus	enterotoxin A, staphylokinase	yes	phages PS42-D, Φ13	Betley & Mekalanos 1985; Winkler *et al.* 1965
Agrobacterium tumefasciens	tumour-inducing DNA	yes	Ti plasmids	Van Larebeke *et al.* 1975
Pseudomonas aeruginosa	cytotoxin	yes	phage ΦCTX	Hayashi *et al.* 1990
Pseudomonas syringae	coronatine phytotoxin	yes	COR plasmids	Bender *et al.* 1989
Bacillus thuringensis	δ-endotoxin	no	pXO plasmids	González *et al.* 1982

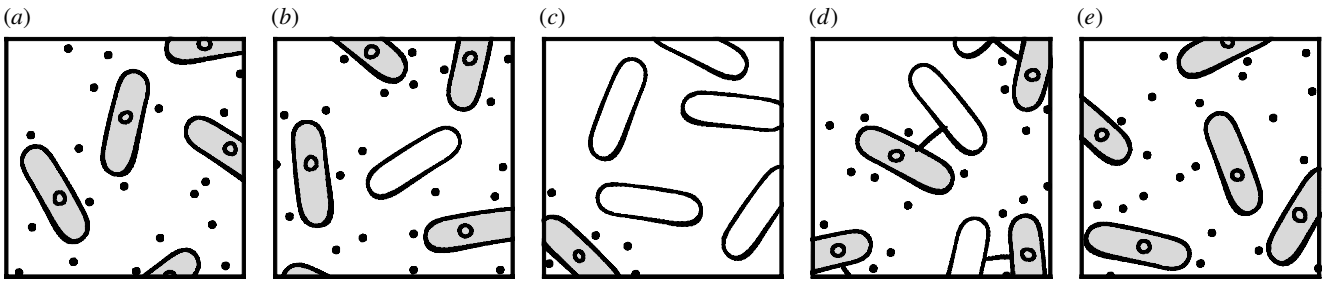

Figure 1. Cheater hypothesis for maintenance of virulence-factor genes on horizontally transmissible elements. (*a*) Pathogenic bacteria (shown in grey) produce an extracellular virulence factor (black dots). (*b*) Mutant cheaters (shown in white) that do not produce the virulence factor can be complemented extracellularly. (*c*) Cheaters increase in frequency because they do not pay the metabolic cost of producing the virulence factor. (*d*) Horizontal transfer (shown here as conjugation) reintroduces functioning virulence-factor genes to cheaters and (*e*) converts them into producers of the virulence factor.

despite low levels of transfer between different chromosomal lineages. I illustrate this hypothesis using simple mathematical models of the population dynamics of bacterial pathogens within and between hosts, discuss how the hypothesis can be tested with simple laboratory experiments, and discuss its implications for the evolution of pathogen virulence and the evolution of cooperative social behaviour.

(a) *Overview of the hypothesis*

The hypothesis is shown schematically in figure 1. If those genes whose products we recognize as virulence factors have evolved to aid bacteria in pathogenic life, their effect must be to increase the pathogen's net rate of infectious transmission. Virulence factors may do this by helping pathogens colonize susceptible hosts, obtain resources and multiply within the host, evade the host's defences or disperse to colonize new hosts. Many virulence factors, however, are secreted by bacteria into their extracellular environment (table 1). The transmission benefits of virulence factors that act extracellularly are potentially available to members of a pathogen population within a host other than those that originally produced the factor. In this case, a rare mutant 'cheater' strain that does not produce the factor, thereby avoiding the metabolic cost of virulence-factor production, can be complemented extracellularly by other members of the population within a host and can increase in frequency over the course of an infection. If pathogens compete for resources within a host, then the ascent of cheaters will reduce infectious transmission of the virulence-factor-producing strain. Such a strain could recover infectiousness lost to cheaters, however, if it could reintroduce functional versions of the virulence-factor genes into cheaters via horizontal transfer and convert them into producers. I propose that virulence-factor genes might be maintained on horizontally transmissible genetic elements by between-host selection to prevent cheating.

2. MODEL AND RESULTS

To illustrate and formalize the above arguments, I use a simple mathematical model describing the within-host

population dynamics of a rapidly reproducing bacterial pathogen that is eventually cleared by the host immune response (modified from Antia *et al.* (1994)). This model is not meant to realistically describe the quantitative details of the life cycle of any specific pathogen. Instead, it is meant to make sure that somewhat vague ideas of fitness and benefit can be translated into systematic changes in the frequencies of alleles in populations due to the differential survival and reproduction of their carriers. I consider the special case in which a virulence factor increases pathogen reproduction within a host by making available some limiting nutrient, as in, for example, liberation of iron by *Escherichia coli* α-haemolysin (Waalwijk *et al.* 1983). I show that horizontal transfer of such virulence-factor genes increases infectious transmission of a virulence-factor-producing strain under conditions favourable to cheaters.

(a) *Within-host population dynamics*

I first consider the case with no horizontal transfer. In the model, the pathogen population within a host consists of two strains, one that produces an extracellular virulence factor and another that does not produce the factor but is otherwise identical. The population size of the virulence-factor-producing strain at a time t since infection is $P(t)$ and that of the non-producing cheater strain is $C(t)$. The total pathogen population is $N(t) = P(t) + C(t)$ and the frequency of the producer strain is $Q(t) = P(t)/N(t)$. Cheaters are assumed to be present in the pathogen population at the time of infection at some low frequency $(1-Q_0)$ due to mutation or co-transmission from the previous host. Throughout this paper I use the subscript zero to indicate a variable's value at $t = 0$.

Rather than explicitly tracking the extracellular concentrations of virulence factor and limiting nutrient, the factor's effect can be simplified to an increase b in the exponential reproductive rate of both producer and cheater strains from a basal rate, r. If $r < 0$, the virulence factor is necessary for growth within a host. Because both pathogen strains can consume the nutrient, the effects of the virulence factor are diluted by the presence of cheaters such that the increase in growth rate is proportional to the frequency of the producing strain (see Appendix A). The benefits of the virulence factor are assumed to be shared equally among all members of the pathogen population but the producing strain pays a metabolic cost such that its reproductive rate is reduced by an amount c relative to cheaters. The virulence factor is assumed to confer a net benefit in the absence of cheaters, such that $b > c$.

The strength of the host's inducible immune response, measured, for example, by the number of activated macrophages and pathogen-specific B and T cells, is $I(t)$. Pathogens are removed by the immune response with a specific killing rate k. The immune response proliferates in proportion to the total pathogen density with saturating kinetics at maximum rate ρ. The pathogen density at which the immune response proliferates at half maximum is ϕ. Decay of the immune response in the absence of the pathogen is assumed to be slow relative to the length of infection and so is not included here. This form for the immune response is a simple way of controlling the pathogen population but its specific details do not affect the main results.

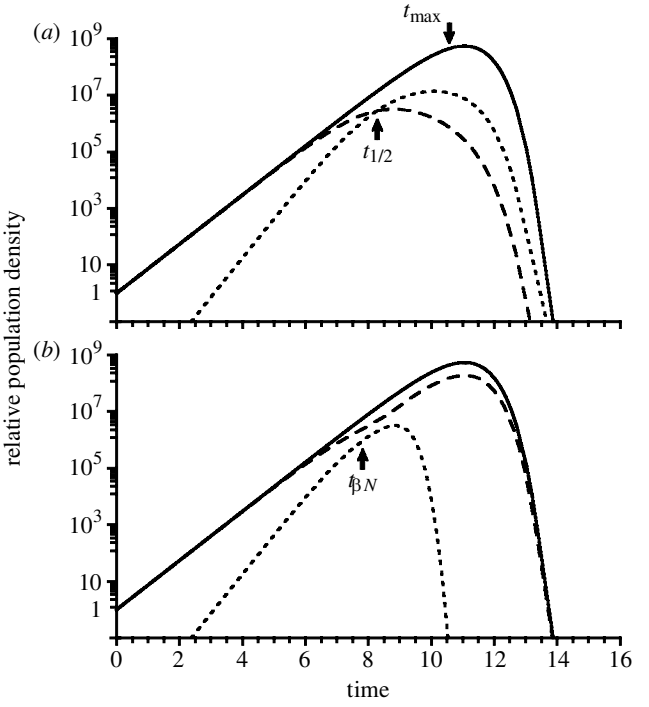

Figure 2. Within-host population dynamics of bacterial pathogens with and without horizontal transfer of virulence-factor genes. (*a*) Horizontal-transfer rate $\beta = 0$. (*b*) $\beta = 2\times10^{-7}$. Solid line, virulence-factor-producing strain in the absence of cheaters; dashed line, producer strain when cheaters are present; dotted line, cheater strain. Arrows indicate $t_{\frac{1}{2}}$, t_{max} and $t_{\beta N}$ (see § 2(b,c) and equations (A6), (A8) and (A9)). For simplicity, populations are measured in units of their initial densities, so that $N_0 = I_0 = 1$. Other parameters are as follows: $r = 0.5$, $b = 2.7$, $c = 1.2$, $k = 10^{-3}$, $\rho = 1.0$, $\phi = 10^3$ and $(1-Q_0) = 5\times10^{-5}$.

With these assumptions, the dynamics of pathogen density and immune response are described by the equations

$$dP/dt = (r + bQ - c)P - kIP, \quad (1)$$

$$dC/dt = (r + bQ)C - kIC, \quad (2)$$

$$dI/dt = \rho IN/(N + \phi). \quad (3)$$

The initial size of the pathogen population is assumed to be much smaller than that which stimulates the immune system, such that $N_0 \ll \phi$. The producer strain's maximum reproductive rate, $(r+b-c)$, and the immune proliferation rate, ρ, are assumed to be similar to within an order of magnitude (Antia *et al.* 1994). The host is assumed to be immunologically naive such that immune-induced pathogen mortality is initially small and $kI_0 \ll (r+b-c)$.

Figure 2*a* shows an example of the population dynamics of infection under this model. In the absence of cheaters, the virulence-factor-producing strain reproduces exponentially for a time until it is eventually cleared by the host immune response. Cheaters, when initially present as a minority subpopulation, increase in frequency as the infection progresses because they benefit from the effects of the extracellular virulence factor without incurring the metabolic cost of its production. As cheaters become common, they dilute the benefits of the

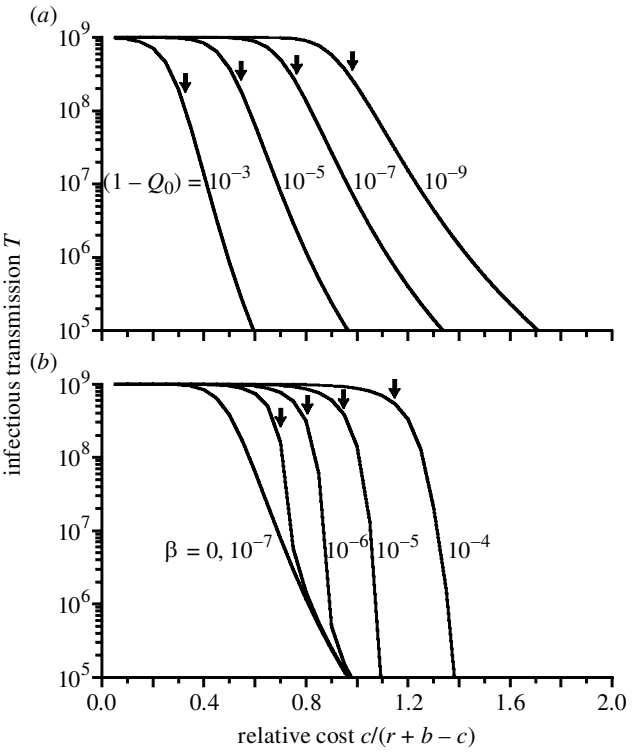

Figure 3. Infectious transmission as a function of the relative cost of virulence-factor production $c/(r+b-c)$ for several values of (*a*) initial cheater frequency, $(1-Q_0)$ and (*b*) horizontal transfer rate β. Horizontal transfer of virulence-factor genes increases infectious transmission at intermediate costs. Arrows in (*a*) indicate minimum cost for which equation (6) is satisfied. Arrows in (*b*) indicate maximum cost for which equation (9) is satisfied. $(r+b-c)=2.0$, $r=0.5$ and $\tau=1$. In (*a*), $\beta=0$. In (*b*), $(1-Q_0)=10^{-5}$. Other parameters are as in figure 2.

virulence factor and in doing so reduce the growth rate of both pathogen populations. This also reduces the peak population size reached by the virulence-factor-producing strain.

To see how these dynamics depend on model parameters, consider an approximate expression for the size of the virulence-factor-producing population over the course of an infection,

$$P(t>t_1) \approx \frac{P_0 e^{(r+b-c)t}}{(Q_0+(1-Q_0)e^{ct})^{b/c}} \exp\left[\frac{kI_1}{\rho}(1-e^{\rho(t-t_1)})\right], \quad (4)$$

where t_1 is the time at which the growing pathogen population reaches a density ϕ and $I_1 = I(t_1)$ (see Appendix A for derivation). The numerator of the first term in equation (4) describes exponential growth of the producer strain under the beneficial effects of the virulence factor. The second term describes the eventual clearance of the pathogen by the host immune response. The denominator of the first term describes the reduction in growth rate of the producer strain associated with the increasing frequency of cheaters. As cheaters become common, growth of the producer strain decreases from a rate $(r+b-c)$ to a net rate $(r-c)$. The reduction in growth rate occurs sooner for greater initial frequencies of cheaters and for greater costs of production of the virulence factor, but is independent of virulence-factor benefits. In this model the virulence-factor-producing strain always decreases in frequency during an infection (see Appendix A).

(b) *Epidemiology and infectious transmission*

Equations (1)–(3) describe the dynamics of a pathogen population within a host, but in order to persist in a population of hosts a pathogen must be infectiously transmitted to at least one other susceptible individual, on average, per infection (Anderson & May 1991). In addition, the pathogen strain that causes the greatest number of secondary infections will, in the absence of multiply infected hosts, drive to extinction all other strains to which it provides immunity (Bremermann & Thieme 1989). Therefore, a complete consideration of pathogen fitness must consider a virulence factor's effect on infectious transmission.

I assume that the rate of infectious transmission is proportional to the number of pathogen cells released from an infected host, and thus proportional to the size of the pathogen population within the host at that time. While this is unlikely to be strictly true for many pathogens, more complicated models involving saturation effects do not give qualitatively different results (Antia *et al.* 1994). The total amount of secondary transmission, T, from a single infected host caused by the virulence-factor-producing strain is then given by the integral of pathogen density over time,

$$T = \int_0^\infty \tau P(t)\,dt, \quad (5)$$

where τ is the average number of infections caused per pathogen cell (Antia *et al.* 1994). Adaptation among endemic pathogens maximizes T with respect to biological constraints on reproduction and transmission (Bremermann & Thieme 1989).

Figure 3*a* shows transmission of the producer strain as a function of the relative cost of virulence-factor production, $c/(r+b-c)$, and initial cheater frequency, $(1-Q_0)$. Relative cost is equivalent to the within-host selection coefficient favouring cheaters. For a given value of $(r+b-c)$, transmission is approximately constant at small costs but falls off exponentially above a certain threshold. This threshold corresponds to those conditions under which cheaters become common enough to significantly reduce the net growth rate of the pathogen population and thus reduce the maximum population density attained by the virulence-factor-producing strain. Since transmission is dominated by those times when pathogen density is highest, the peak density attained within a host is a major determinant of pathogen fitness.

While equation (5) is not amenable to analytical solution, an approximate condition for when cheaters become common enough to substantially reduce transmission can be found by comparing the time at which $Q=0.5$, $t_{\frac{1}{2}}$, to the time at which the producer strain reaches a peak density in the absence of cheaters, $t_{\max}$. Examples of $t_{\frac{1}{2}}$ and $t_{\max}$ are indicated in figure 2*a*. Cheaters substantially reduce transmission if $t_{\frac{1}{2}} < t_{\max}$ or, equivalently,

$$(1-Q_0)^{\rho/c}\frac{r+b-c}{kI_0}\left(\frac{\phi}{eN_0}\right)^{\rho/(r+b-c)}>1 \qquad (6)$$

(see Appendix A for derivation). Equation (6) shows that cheaters are more likely to reduce transmission of the producer strain for greater costs of virulence-factor production and for greater initial cheater frequencies, as shown in figure 3*a*. In addition, cheaters are more likely to reduce transmission in infections of longer duration (caused, for example, by decreased k or increased ϕ). This occurs because longer infections allow more time for cheaters to become common enough to reduce the growth rate of the producer strain. Arrows in figure 3*a* indicate the minimum values of $c/(r+b-c)$ for which equation (6) is satisfied.

A virulence-factor-producing strain can be maintained in a population of hosts despite interference from cheaters if the pathogen is sufficiently infectious and the virulence factor is sufficiently beneficial. Between-host epidemiological models similar to those described by Bonhoeffer & Nowak (1994*a*) show that producers can be maintained as long as their basic reproductive number, R_0 (Anderson & May 1991), is not reduced below 1 or below that of the cheater strain alone (J. Smith, unpublished data). The former condition will depend on the density of hosts and the infectiousness of the pathogen, while the latter will depend on the relative values of r and $(r+b-c)$. If producers are maintained, then cheaters will also be maintained at some frequency due to recurrent within-host selection. It is also interesting to note that if the frequency of cheaters in an initial inoculum is correlated with their frequency in the previous infected host, then cheater interference with infectious transmission can be compounded from one infection to the next.

(c) *Virulence-factor mobility*

Now consider a virulence factor the genes of which can be transmitted horizontally between pathogen strains within a host through the action of conjugative plasmids or lysogenic phages. I assume that horizontal transfer has mass-action kinetics with transfer rate β, proportional to the product of producer and cheater densities. Accordingly, equations (1) and (2) are replaced by

$$\mathrm{d}P/\mathrm{d}t=(r+bQ-c)P-kIP+\beta PC, \qquad (7)$$

$$\mathrm{d}C/\mathrm{d}t=(r+bQ)C-kIC-\beta PC. \qquad (8)$$

This simple expression for horizontal transfer, based on infectious transmission in epidemiological models (Anderson & May 1991), has been shown to describe accurately the dynamics of plasmid transfer despite the fact that it ignores potential complications such as latent periods and saturation effects (Simonsen *et al.* 1990). For lysogenic phage it subsumes phage production, degradation and lysogeny into a single parameter.

Figure 2*b* shows an example of the within-host population dynamics of infection when virulence-factor genes are horizontally transmissible. As before, cheaters initially increase in frequency as the infection progresses and begin to slow the growth of the producer strain. Once the pathogen population reaches a density where $\beta N>c$, however, the net rate of horizontal transfer exceeds the metabolic cost of the virulence factor and cheaters decline rapidly in frequency as they acquire virulence-factor genes and become producers (see Appendix A). This allows the virulence-factor-producing population to reach a peak density nearly high as it would have been in the absence of cheaters. Due to the mass-action kinetics, horizontal transfer is most effective at reducing cheater frequency at high pathogen densities, which is when most infectious transmission occurs.

Figure 3*b* shows infectious transmission for different values of the horizontal transfer rate, β. The main effect of horizontal transfer is to increase the threshold cost above which cheaters substantially reduce infectious transmission. There is thus a range of intermediate virulence-factor costs for which pathogens with horizontally transmissible virulence-factor genes cause many more secondary infections than those with non-mobile genes. It is in this region that horizontal transfer can be maintained by between-host selection to prevent cheating.

An approximate measure of the parameter range over which horizontal transfer is effective can be found by comparing the time at which a cheater-free population reaches a density where $\beta N>c$, $t_{\beta N}$, to the time $t_{\frac{1}{2}}$ described above. Horizontal transfer is effective in preventing cheaters from interfering with infectious transmission if $t_{\beta N}<t_{\frac{1}{2}}$, or equivalently,

$$(1-Q_0)^{1/c}\left(\frac{c}{\beta N_0}\right)^{1/(r+b-c)}<1 \qquad (9)$$

(see Appendix A). Equation (9) shows that horizontal transfer is more effective for greater transfer rates, smaller metabolic costs and smaller initial cheater frequencies. It is also more effective for greater exponential growth rates, which allow the pathogen population to reach more quickly the critical within-host density where the net transfer rate exceeds the metabolic cost of the virulence factor. Arrows in figure 3*b* indicate the maximum values of $c/(r+b-c)$ for which equation (9) is satisfied. Thus, if equation (6) is satisfied such that cheaters reduce the fitness of a virulence-factor-producing pathogen, then selection will maintain horizontal transmission of virulence-factor genes if equation (9) is satisfied as well.

3. DISCUSSION

Using simple mathematical models, I have shown that selection on pathogens for infectious transmission between hosts can maintain virulence-factor genes on horizontally transmissible genetic elements if mutant pathogen strains can reap the benefits of the virulence factor without paying its metabolic cost. Horizontal transfer forces would-be cheaters to produce the virulence factor. Since horizontal transfer would occur primarily between a pathogen strain and its cheating derivatives, this hypothesis provides a mechanism for the maintenance of virulence-factor mobility despite low levels of transfer between separate bacterial ecotypes (Karaolis *et al.* 1995; Beltrán *et al.* 1999).

I have modelled only the special case of pathogens that cause acute infection and factors that increase within-host

growth but there is no obvious reason why the cheater hypothesis would not also apply to pathogens that cause persistent infections or to other types of virulence factors, such as those that aid in colonization of hosts or those that increase the infectiousness of released pathogen cells. Since extracellular complementation is potentially a very general phenomenon, this hypothesis can potentially explain the maintenance of horizontal transfer in many different pathogen species and for many different virulence factors. Indeed, the cheater hypothesis may also apply to non-virulence traits. This could include, for example, symbiotic nitrogen fixation in *Rhizobia* spp. where cheaters that do not fix nitrogen could still benefit from plant exudates (Van Rhijn & Vanderleyden 1995), antibiotic-resistance genes where cheaters that remain sensitive could still benefit from a detoxified local environment (Lundbäck & Nordström 1974), and anti-competitor bacteriocins where cheaters that do not synthesize the bacteriocin could still retain immunity (Durrett & Levin 1997).

(a) *Predictions and empirical tests*

The cheater hypothesis makes a number of testable predictions. First, it predicts that the virulence factors carried by horizontally transmissible elements are those for which virulence-factor-defective mutants can be complemented extracellularly. This can be tested with simple co-infection experiments. Such cheating has been experimentally observed among toxins (Waalwijk *et al.* 1983), siderophores (Wolf & Crosa 1986), type III secretion systems (Hirano *et al.* 1999) and even some pili (Hammar *et al.* 1996; Wall *et al.* 1998). Second, the hypothesis predicts that there is a significant metabolic cost to virulence-factor production. Such a cost can be measured *in vivo* by the ability of cheaters to increase in frequency when rare (e.g. Hirano *et al.* 1999) or *in vitro* with competition experiments under laboratory conditions that induce production of the virulence factor. Finally, the hypothesis predicts that cheaters reduce the fitness of producing strains when the virulence factor is non-transmissible and that horizontal transfer reduces the fitness burden of cheaters. This can be tested if there exists a suitable experimental model for infectious transmission between hosts or by the ability of cheaters to dilute within-host benefits.

Note that support for these predictions would not rule out the possibility that other mechanisms may also be involved in maintaining horizontal transfer of virulence-factor genes. Empirical support would, however, reduce the need to invoke other mechanisms. For example, generation of novel pathogen strains through horizontal gene transfer (Karaolis *et al.* 1995; Whittam 1998) may simply be a coincidental side-effect of virulence-factor mobility.

(b) *Genetic regulation of horizontal transfer*

The cheater hypothesis accounts for the fact that horizontal transfer is sometimes genetically coordinated with virulence-factor expression, as seen in the tumour-inducing plasmids of *Agrobacterium* spp. (Winans *et al.* 1999), the Shiga toxin phages of *E. coli* (Neely & Friedman 1998) and the cholera toxin phages of *V. cholerae* (Lazar & Waldor 1998). If horizontal transfer itself has some metabolic cost, then there would be selection to only express transfer functions when cheaters are likely to be present. Since cheaters only increase in frequency by foregoing the metabolic cost of virulence-factor production, this would be when the virulence factor is being expressed. In *Agrobacterium*, horizontal transfer is also correlated with high pathogen density via a plasmid-borne quorum-sensing mechanism, a trait some have found puzzling (Winans *et al.* 1999). Under the cheater hypothesis, however, this can be seen as a mechanism for restricting transfer to those times when it is effective in reducing the frequency of cheaters, that is, only when the threshold condition $\beta N > c$ is met (figure 2*b*).

(c) *Problems and limitations*

The simple model presented here ignores a number of complicated realities that pose significant challenges to the hypothesis. The first concerns the origin of cheaters. The model assumes that cheaters are present at some low frequency in the pathogen population at the beginning of an infection and includes no explicit term for their generation by mutation or loss of the mobile genetic element. This assumption is appropriate if the rate of generation of cheaters is small relative to the initial pathogen population size and to the metabolic cost of the virulence factor. For many pathogens, however, the initial infecting population can be very small, as few as ten cells for *Shigella dysenteriae* (DuPont *et al.* 1989). In such a case, cheaters would be entirely absent at first and not generated by mutation until some time later, when the population reaches a considerable size. This would lengthen the time required for cheaters to reach an appreciable frequency and would thus reduce the strength of selection for horizontal transfer.

Another concern is the genetics of cheating. Plasmids and phage often carry genes that inhibit super-infection by closely related elements (Ippen-Ihler & Skurray 1993; Ptashne 1992). The rate of transfer of virulence-factor genes to cheaters that retain these exclusion functions will be much less than for cheaters generated by the loss of the entire element. Similarly, cheaters could also evolve resistance to horizontal transfer. Thus, the effectiveness of horizontal transfer in insuring all pathogen cells produce a virulence factor depends sensitively on the genetic characteristics of cheaters that affect transfer rates.

There is also the potentially confounding factor of spatial structure within hosts. The model assumes that virulence-factor benefits are equally available to all pathogen strains within a host. In reality, pathogen cells may be clustered in microcolonies consisting of a single clone, such that the benefits of a secreted virulence factor may be preferentially experienced by clone-mates of the producing cells. This would reduce within-host selection for cheaters and subsequent selection for horizontal transfer.

Finally, the hypothesis only applies to virulence factors that increase pathogen fitness. It can thus explain the horizontal transfer of factors that increase colonization or infectious transmission, but not of factors that affect only the severity of the disease. The putative fitness benefits of many virulence factors are unknown.

(d) *Pathogen virulence and social evolution*

Infection by multiple pathogen strains is often claimed to promote the evolution of increased virulence,

increasing the relative value of within-host reproduction at the expense of host survival (Herre 1993; Nowak & May 1994; Frank 1996; Ebert & Mangin 1997). These studies assume that pathogen strains within a host are engaged in scramble competition for host resources and that virulence is a by-product of pathogen replication. For some pathogens, however, within-host reproduction may be a cooperative activity, involving secreted factors whose benefits are shared among pathogen strains (e.g. Bonhoeffer & Nowak 1994*b*). If virulence is primarily a consequence of these factors, then infection by multiple pathogen strains could promote the spread of cheaters and the evolution of decreased virulence. The cheaters would, in effect, act as hyperparasites (Taylor *et al.* 1998).

As protection against cheaters, horizontal transfer of genes for cooperative traits is a novel mechanism for the evolutionary maintenance of cooperation. This mechanism is distinct from (but complementary to) others such as kin selection (Hamilton 1964), intrademic group selection (Wilson 1975; Dugatkin & Reeve 1994), reproductive bribing (Reeve & Keller 1997), reciprocity (Trivers 1971) and policing (Frank 1995). For obvious reasons, however, horizontal transfer is a cooperative mechanism that is probably only available to bacteria and other unicellular micro-organisms. This mechanism also suggests that plasmids and phage have been co-opted to maintain cooperation among the very bacterial cells they originally evolved to parasitize.

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APPENDIX A

The simple bQ term in equations (1) and (2) can be derived from a model with explicit variables for the concentrations of an extracellular virulence factor, V, and a limiting nutrient, S, that the virulence factor makes available. Instead of bQ, the growth rates of both producers and cheaters are increased by amount α proportional to nutrient concentration. The virulence factor is produced by the producing strain at a rate σ and degraded at a rate μ. Nutrients are liberated at rate ω, proportional to virulence-factor concentration, and are consumed by both pathogen strains at a rate ε. Thus,

$$\mathrm{d}P/\mathrm{d}t = (r + \alpha S - c)P - kIP, \tag{A1}$$

$$\mathrm{d}C/\mathrm{d}t = (r + \alpha S)C - kIC, \tag{A2}$$

$$\mathrm{d}V/\mathrm{d}t = \sigma P - \mu V, \tag{A3}$$

$$\mathrm{d}S/\mathrm{d}t = \omega V - \varepsilon(P + C)S. \tag{A4}$$

If the dynamics of virulence factor and nutrient concentration are much faster than those of the pathogen population, then at the quasi-steady state $V = \sigma P/\mu$ and $S = \sigma\omega Q/\mu\varepsilon$. Using these values and defining $b = \alpha\sigma\omega/\mu\varepsilon$, equations (A1) and (A2) reduce to equations (1) and (2).

The change in frequency of the virulence-factor-producing strain under equations (1) and (2) is $\mathrm{d}Q/\mathrm{d}t = -Q(1-Q)c$, which has the solution

$$Q(t) = Q_0/(Q_0 + (1-Q_0)\mathrm{e}^{ct}). \tag{A5}$$

Since $\mathrm{d}Q/\mathrm{d}t$ is always negative and has equilibria only at $Q = 1$ or 0, the producer strain always decreases in frequency over the course of an infection where cheaters are present. From equation (A5), the time at which $Q = 0.5$ is

$$t_{\frac{1}{2}} = \frac{1}{c}\ln\left(\frac{Q_0}{1-Q_0}\right). \tag{A6}$$

An approximate expression for the density of the producer strain can be found by separating the course of infection into two regions (Antia *et al.* 1994). Region (*i*) is defined as that time $t < t_1$ where $N \leqslant \phi$. In this region I make the approximations $N \ll \phi$, $kI \ll (r + b - c)$ and $(1-Q) \ll 1$. The rates of change of pathogen and immune response are then approximately $\mathrm{d}N/\mathrm{d}t \approx (r + b - c)N$ and $\mathrm{d}I/\mathrm{d}t \approx \rho IN/\phi$, which have the solutions $N(t < t_1) \approx N_0\mathrm{e}^{(r+b-c)t}$ and $I(t < t_1) \approx I_0\mathrm{e}^{\rho(N-N_0)/(r+b-c)\phi}$. Thus, $t_1 \approx [\ln(\phi/N_0)]/(r + b - c)$ and $I_1 \approx I_0\mathrm{e}^{\rho/(r+b-c)}$. Region (*ii*) is defined as $t > t_1$, where I make the approximation $N \gg \phi$ such that $\mathrm{d}I/\mathrm{d}t \approx \rho I$ and

$$I(t > t_1) \approx I_1\mathrm{e}^{\rho(t-t_1)}. \tag{A7}$$

Equation (4) can be obtained by substituting Q and I from equations (A5) and (A7) into equation (1) and then integrating from t_1 to t. The approximate time at which a virulence-factor-producing pathogen reaches a peak density in the absence of cheaters, $t_{\max}$, can be found by setting equation (1) equal to zero, setting $Q = 1$, substituting I from equation (A7) and solving for t,

$$t_{\max} \approx \frac{1}{\rho}\ln\left[\frac{r + b - c}{kI_0}\left(\frac{\phi}{\mathrm{e}N_0}\right)^{\rho/(r+b-c)}\right]. \tag{A8}$$

With horizontal transfer of virulence-factor genes, the change in frequency of the producer strain under equations (7) and (8) is $\mathrm{d}Q/\mathrm{d}t = Q(1-Q)(\beta N - c)$. Thus, cheaters decrease in frequency when $\beta N > c$. The approximate time at which a growing pathogen population reaches a density where $\beta N > c$ in the absence of cheaters $t_{\beta N}$, can be found by substituting $N(t) \approx N_0\mathrm{e}^{(r+b-c)t}$ into $\beta N = c$ and solving for t,

$$t_{\beta N} \approx \frac{1}{r + b - c}\ln\left(\frac{c}{\beta N_0}\right). \tag{A9}$$

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