

Plasmids survive despite their cost and male-specific phages due to heterogeneity of bacterial populations

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ABSTRACT

Problem: Conjugative plasmids manipulate their bacterial hosts to express sex-pili – long, filamentous organelles whose genes are carried by the plasmids themselves. Sex-pili locate and attach to another bacterial cell for plasmid transfer. Bacteria differ in their ability to express sex-pili and hence to donate plasmids. Does this diversity in sex-pilus expression contribute to the survival of conjugative plasmids?

Method: Simulations of a chemostat containing bacterial cells, some harbouring a conjugative plasmid. Certain viruses, called male-specific phages (MSPs), enter the chemostat. These viruses infect only donor cells. I monitor whether plasmids continue to survive in each simulation.

Key assumption: A bacterium's ability to donate is proportional to its susceptibility to MSPs.

Conclusions: Bacterial diversity in sex-pilus expression strongly increases the size of the parameter space for plasmid survival. A host with low expression of sex-pili – and hence a low plasmid transfer rate – represents a sink habitat for the conjugative plasmid. However, hosts with high expression of sex-pili also constitute sink habitats – although they have a high plasmid transfer rate, they are the most susceptible to MSPs. A system consisting of both types of bacteria (two sink habitats) constitutes a source habitat for the conjugative plasmid. At the same time, this diversity helps to maintain the MSP itself because extinction of the conjugative plasmids would also doom the MSP.

Keywords: maintenance of plasmids, male-specific-phages, polymorphism, sink habitat, source habitat.

INTRODUCTION

In addition to their chromosomal DNA, many bacterial cells bear one or more plasmidic DNA molecules. For example, in *Escherichia coli* isolated from the faeces of a single human host, Caugant *et al.* (1981) found that 50 of 53 electrophoretic types carried at least one plasmid, and 42 appeared to carry more than one. In another study, Sherley and colleagues

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(2003) examined the plasmid content of 223 wild-type enterobacteria isolated from 223 wild mammals, and found that 63% of these strains carry one or more plasmids. Clearly, this is an underestimate given that the authors only looked for plasmids belonging to five plasmid groups (incompatibility groups), which is only a fraction of the total number of incompatibility groups (Couturier *et al.*, 1988; Summers, 1996).

To be maintained in a given bacterial lineage, plasmids have to replicate themselves at least once in every bacterial division. Moreover, plasmid replication has to be frequent enough in each bacterial division to impede segregation, in which, upon bacterial division, one daughter cell receives no plasmid. Plasmids have different ways to control copy number or to ensure stable plasmid inheritance. All these processes are expected to confer a fitness cost to plasmid-bearing bacteria.

For a given plasmid–bacterium dyad to cope with these costs, either the plasmid confers an advantage to its host, or the plasmid is able to transfer fast enough to plasmid-free cells. For example, many plasmids carry genes conferring resistance to antibiotics (Meynell *et al.*, 1968), to pollutants or to UV light (Perry and Walker, 1982), as well as virulence factors that enable bacteria to infect higher organisms (Martinez and Baquero, 2002); other functions are more subtle, such as to help biofilm formation (Ghigo, 2001). However, even if plasmids carry these advantageous genes, it may still constitute a paradox that these genes have not been integrated into the chromosome (Bergstrom *et al.*, 2000).

Using mathematical mass action models, Stewart and Levin (1977) concluded that the necessary conditions for conjugative plasmids to be maintained by transfer alone are very broad: bacterial densities just have to be sufficiently high to compensate for plasmid loss by segregation and the cost of bearing the plasmid. However, given the parameters obtained experimentally with *E. coli*, it was realized that the necessary conditions for conjugative plasmid maintenance are not fulfilled (Levin *et al.*, 1979; Gordon, 1992).

Meanwhile, other solutions to this problem arose. The mathematical models so far described can be regarded as a ‘null hypothesis’, given that the assumptions of the models did not consider some potentially important features of plasmids. Transitory derepression, where for a period of time after the receipt of a plasmid conjugative pili synthesis is derepressed in the transconjugant and its immediate descendants, is one of these features. Derepression of sex-pili synthesis leads to a strong increase in the ability of plasmids to transfer (Lundquist and Levin, 1986; Willetts, 1974). However, not all conjugative plasmids are able to transiently derepress sex-pili synthesis; one of these plasmids is, for example, the R1 plasmid, an F-type resistant plasmid isolated more than 40 years ago. So this plasmid constituted the focus of further study and it was found that the R1 plasmid has different transfer rates among different enterobacterial strains and that these rates span at least six orders of magnitude (Gordon, 1992; Dionisio *et al.*, 2002). Although the R1 plasmid has a low transfer rate in most of the strains, Dionisio *et al.* (2002) found two exceptions: when the R1 plasmid is in a given strain of *Erwinia chrysanthemi* or in one of the *E. coli* strains (a natural isolated strain collected in Mali), its transfer rate is much higher than in other strains (Dionisio *et al.*, 2002). That is, these two strains constitute excellent donors and recipients of the R1 plasmid. Therefore, these cells can help the plasmid to spread towards cells of other strains, given that after spreading among the good donors, these populations of transconjugants may now re-transfer the R1 plasmid to cells of other co-inhabiting strains (Dionisio *et al.*, 2002).

Nevertheless, the conjugative plasmids expressing very high levels of sex-pili become extremely sensitive to phages (bacteria-infecting viruses) that infect bacteria precisely

through these sex-pili – the male-specific phages (MSPs) (Anderson, 1968; Campbell, 1996). There are various and unrelated MSPs, such as the single-stranded DNA phage M13 or the RNA phage MS2 (Ackermann and DuBow, 1987; Campbell, 1996). MS2 phages, the focus of this paper, lyse bacterial cells after 20 min of infection, producing more than 10,000 phages ready to infect other cells (Campbell, 1996).

Naturally occurring conjugative plasmids have evolved mechanisms to repress the synthesis of conjugative sex-pili, thus reducing their rate of horizontal transfer (Gasson and Willetts, 1975; Gaffney *et al.*, 1983). The repression mechanisms are coded by the plasmids themselves. It was proposed long ago by Anderson (1968) that repression reduces the energetic costs to the host resulting from the constitutive expression of the plasmid transfer genes, and lowers the risk of attack by MSPs.

In summary, when plasmids strongly induce the expression of sex-pili in the membrane of their bacterial hosts, plasmids increase their chances of horizontal transmission but they also increase the risk of attack by MSPs. Based on the analysis of a mathematical model of the population dynamics of bacteria, conjugative plasmids and phages in chemostats, I show that the parameter space allowing the maintenance of conjugative plasmids is very narrow. Then I show that diversity in donor ability among bacteria due to strain variety (Gordon, 1992; Dionisio *et al.*, 2002) or to transitory derepression of plasmids, increases the space of parameters leading to the maintenance of conjugative plasmids.

MODELS

The models presented in this section are adapted mainly from two previously described models. Both represent bacteria growing in chemostat habitats. One describes the transmission of conjugative plasmids among bacteria (Stewart and Levin, 1977; Levin *et al.*, 1979) and the other describes lytic phages infecting susceptible bacteria (Levin *et al.*, 1977; Lenski, 1988). Several parameters used in the simulations were obtained experimentally (see Levin *et al.*, 1979; Lenski 1988; Gordon 1992; Campbell, 1996; Dionisio *et al.*, 2002), using *Escherichia coli* strains, plasmids R1, R1drd19 and F, and the MS2 phage. Plasmids R1, R1drd19 and F belong to the Inc-F group, which is a group of plasmids commonly found among enterobacteria. The R1drd19 plasmid is isogenic to the R1 plasmid but it constitutively expresses sex-pili because the genes normally responsible for repression are mutated (Meynell and Datta, 1967; Koraimann *et al.*, 1996). The F plasmid also constitutively expresses sex-pili due to mutations in the repression system (Finlay *et al.*, 1986). The virus MS2 is an RNA MSP with 3569 nucleotides. It infects bacteria using the F pilus as a receptor, its burst size is 10^4 particles within 22 min of latency, and a sole holin is responsible for lysis (for a review, see Campbell, 1996).

Unless otherwise stated, I used the values presented in Table 1 in the simulations reported in this paper.

The aim of this paper is to analyse the effect of bacterial diversity on plasmid maintenance. Therefore, I have to consider the existence of two bacterial ‘types’, X and Y (if they are plasmid-free), and X_p and Y_p (if they bear the conjugative plasmid). In modelling two co-inhabiting strains, the two ‘types’ correspond to the two strains: one in which plasmid-bearing cells strongly express sex-pili (hence a high transfer rate), and another in which sex-pili expression is weak (hence a low transfer rate). Though in modelling transitory derepression I consider only one strain (X cells), plasmid-bearing cells may present two different phenotypes in terms of sex-pili expression that translate into high and low plasmid transfer rates. Cells that acquired the plasmid many generations before have

a low expression of sex-pili (repressed conjugation), whereas cells that recently acquired the plasmid have a high expression of sex-pili [transitory derepression (see Lundquist and Levin, 1986)].

In general, I consider a chemostat containing a population of plasmid-free and plasmid-bearing bacteria (infected or not infected by the phage MS2), free MS2 phage particles, and a potentially limiting bacterial resource. The habitat is liquid and well mixed, such that bacteria, phages and resources encounter one another at random. The resource has a concentration C_0 ($\text{mg}\cdot\text{l}^{-1}$) as it flows into the habitat at a rate ω (turnovers per hour). Bacteria bearing or not bearing the plasmid, bacteria-infected phages (I), free phages (P), utilized and unutilized resources are washed out of the habitat at this same rate ω .

Bacteria multiply via binary fission and, throughout this paper, it is assumed that bacteria multiply at a per capita rate that is a hyperbolic function of the resource concentration in the habitat (C), the Monod function $M = C/(C + Q)$ (Monod, 1942; Dykhuizen and Hartl, 1983). The maximum specific growth rate is ψ (h^{-1}), and the resource concentration at which the bacteria grow at half this maximum rate is Q ($\text{mg}\cdot\text{l}^{-1}$). Each replication event of a bacterium uses up ε (mg) of the resource. In the model in which there are two different co-inhabiting strains, and to prevent the effect of competition for nutrients between the two bacterial types, we consider the existence of two different resources in the medium, C_x and C_y , each specific for the X or X_p and Y or Y_p bacterial types, respectively. I make this assumption because, when two co-inhabiting populations compete for the same nutrient (i.e. they *occupy* the same ecological niche), everything else being similar, one of the populations may be taken to extinction (Hansen and Hubell, 1980). For the model of transitory derepression, this is not a problem given that all cells (eventually carrying the plasmid) belong to the same strain.

Plasmid-mediated bacterial conjugation is a replicative process, implying that both the donor and the transconjugant behave as plasmid donors after a conjugation event (Summers, 1996). The conjugational transfer rate of the plasmid is considered to be proportional to the product of densities of donors and recipients. For example, the transfer rate between X_p - and X -type cells is given by $\gamma_{xx} \cdot X \cdot X_p$ (Stewart and Levin, 1977). Plasmid segregation is proportional to the amount of cells harbouring the plasmid. Its proportionality constant is α .

There is no transfer of a conjugative plasmid towards a cell already bearing a copy of that plasmid – that is, there is no transfer from X_p to X_p cells (Willetts and Maule, 1974; Summers, 1996). Given that there is a fitness cost associated with the presence of a plasmid, the growth rate of these bacterial cells is ψ_p , defined as: $\psi_p \equiv \psi(1 - s)$, with $0 \leq s \leq 1$.

The number of phages that encounter and adsorb to uninfected plasmid-bearing bacteria is a linear function of bacterial and phage densities (Levin *et al.*, 1977). The adsorption constant δ ($\text{ml}\cdot\text{h}^{-1}$) corresponds to the efficiency of each phage to adsorb to plasmid-bearing cells. Each infection by the MS2 phage is lethal to a bacterium (Campbell, 1996), and each infected bacterial cell yields β phage progeny after a latent period of τ (hours), a phenomenon called ‘lysis’.

As explained earlier, sex-pili are coded in conjugative plasmids, and thus MSPs only infect bacterial cells harbouring conjugative plasmids. Therefore, an important assumption is made here: if cells of a given strain express many sex-pili, the adsorption constant δ is higher than if cells express a low number of sex-pili. If there is no conjugative plasmid inside a bacterial cell, the adsorption constant δ is zero. Accordingly, in this paper I assume

that plasmid-bearing cells that are very efficient plasmid donors (due to strong expression of sex-pili) are also highly susceptible to MSPs. The parameter γ_{zz} is the proportionality constant for the transfer rate between two cells of a given Z strain (that is, from Z_p cells to Z cells), and for the rest of this paper I consider γ_{zz} to be proportional to δ_z , the adsorption rate of a given MSP to plasmid-bearing Z cells:

$$\gamma_{zz} = k\delta_z \quad (1)$$

To what value should the parameter k be set? Obviously, k depends on the bacterial strain, on the plasmid and on the MSP. In this paper, I consider values typical for the MS2 phage and IncF plasmids (such as the plasmids F and R1). By inspection and comparison of values described in the literature for the plasmid F, which is permanently derepressed with transfer rate between *E. coli* K12 cells of $\gamma_{K12, K12} \approx 10^{-7} \text{ ml} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$, the measured δ for the male-specific phage MS2 is $\delta \approx 10^{-11} \text{ ml} \cdot \text{h}^{-1}$ (Lenski, 1988). Therefore, we set $k \approx 10^4 \text{ cell}^{-1}$.

In the following two models, X'_p , Y'_p and P' represent the concentrations (per ml), respectively, of plasmid-bearing bacteria not infected by the phage, and free phages, at time $t - \tau$. The function $e^{-\omega \cdot \tau}$ is the fraction of bacteria infected at time $t - \tau$ that has not washed out of the habitat before lysing. The equations differ from the Lotka-Volterra predator equations due to this time lag between entry and death, $t - \tau$, and because the bacterial growth rate is a function of a potentially limiting resource (here assumed to be the Monod function) (Lenski, 1988).

Model I: Differences in sex-pili expression intensity in two co-inhabiting bacterial strains

The equations describing the two co-inhabiting bacterial strains bearing or not bearing the plasmid in the presence of MSPs are as follows:

$$\dot{P} = (\delta_x X'_p + \delta_y Y'_p) \beta P' \cdot e^{-\omega \cdot \tau} - (\delta_x X_p + \delta_y Y_p) P - \omega P \quad (2)$$

$$\dot{I} = -(\delta_x X'_p + \delta_y Y'_p) P' \cdot e^{-\omega \cdot \tau} + (\delta_x X_p + \delta_y Y_p) P - \omega I \quad (3)$$

$$\dot{X} = \psi X M_x - X(\gamma_{xx} X_p + \gamma_{yx} Y_p) - \omega X + \alpha X_p \quad (4)$$

$$\dot{Y} = \psi Y M_y - Y(\gamma_{xy} X_p + \gamma_{yy} Y_p) - \omega Y + \alpha Y_p \quad (5)$$

$$\dot{X}_p = \psi_p X_p M_x + X(\gamma_{xx} X_p + \gamma_{yx} Y_p) - \delta_x X_p P - \omega X_p - \alpha X_p \quad (6)$$

$$\dot{Y}_p = \psi_p Y_p M_y + Y(\gamma_{xy} X_p + \gamma_{yy} Y_p) - \delta_y Y_p P - \omega Y_p - \alpha Y_p \quad (7)$$

$$\dot{C}_x = \omega(C_0 - C_x) - \varepsilon_x(\psi X + \psi_p X_p) M_x \quad (8)$$

$$\dot{C}_y = \omega(C_0 - C_y) - \varepsilon_y(\psi Y + \psi_p Y_p) M_y \quad (9)$$

The dot ($\dot{}$) over P , I , X , X_p , \dots , and C_y , represents the derivative of the respective concentration with respect to time (rate of change). Here, δ_x and δ_y represent the adsorption rates of the phage to plasmid-bearing bacteria (X_p and Y_p , respectively). In these equations, M_x and M_y represent the Monod functions for strains X and Y respectively: $M_x = C_x / (C_x + Q)$ and $M_y = C_y / (C_y + Q)$. I considered that the fitness cost of bearing the plasmid, s , and the growth rate of the two bacterial strains are equal: $\psi_x = \psi_y \equiv \psi$ and $\psi_{px} = \psi_{py} \equiv \psi_p \equiv \psi(1 - s)$.

Model II: Transitory derepression

The equations for transitory derepression of sex-pili expression are as follows:

$$\dot{P} = (\delta_x X_p' + \delta_y Y_p') \beta P' \cdot e^{-\omega \cdot \tau} - (\delta_x X_p + \delta_y Y_p) P - \omega P \quad (10)$$

$$\dot{I} = -(\delta_x X_p' + \delta_y Y_p') P' \cdot e^{-\omega \cdot \tau} + (\delta_x X_p + \delta_y Y_p) P - \omega I \quad (11)$$

$$\dot{Y} = \psi Y M - Y(\gamma_{xy} X_p + \gamma_{yy} Y_p) - \omega Y + \alpha(X_p + Y_p) \quad (12)$$

$$\dot{Y}_p = \psi_p Y_p M + m \cdot Y_p - \delta_y Y_p P - \omega Y_p - \alpha Y_p \quad (13)$$

$$\dot{X}_p = \psi_p X_p M + Y(\gamma_{xy} X_p + \gamma_{yy} Y_p) - m \cdot Y_p - \delta_x X_p P - \omega X_p - \alpha X_p \quad (14)$$

$$\dot{C} = \omega \cdot (C_0 - C) - \varepsilon[\psi_p(X_p + Y_p) + \psi \cdot Y] M \quad (15)$$

In this model, there are plasmid free cells (Y), repressed (Y_p) and derepressed (X_p) plasmid-bearing cells. Derepressed cells are more sensitive to MSPs than repressed cells. Derepression is transitory, and the role of the parameter m corresponds to that of Lundquist and Levin (1986): all newly formed transconjugants enter the plasmid-bearing derepressed population, X_p , but members of this population become repressed and enter the population of repressed plasmid-bearing cells, Y_p at a rate m (h^{-1}) (Lundquist and Levin, 1986). It is further assumed that the fitness costs of bearing a repressed or derepressed plasmid are similar.

Simulations: initial conditions and the addition of MSPs

To determine in which conditions plasmids are maintained upon the sudden occurrence of MSPs, the simulations were performed as follows.

First, I used equations (2) to (9) or equations (10) to (16) to find the equilibrium value of the nutrients, plasmid-bearing and plasmid-free bacterial cells inside the chemostat before the presence of phages. So, in the equations, $P(t) = I(t) = P_0 = I_0 = 0$.

The second step is the addition of MS2 phages to the system. One phage would be enough, but most of the time, and due to the chemostat flow, that phage is usually lost even before infecting a cell. On the other hand, one could consider the introduction in the system of a cell already infected by the phage *just before* lysis. That would release 10^4 phages (see Table 1) and then the problem of random loss of phages would be solved. Therefore, I decided to introduce an intermediate number of phages somewhere between those extreme values: 1000 phages.

Stochastic simulations

The equations were solved numerically by transforming derivatives in ratios of differences and then proceeding to the calculation of concentrations of nutrients, bacteria or phages, in a deterministic way using the respective ordinary differential equations. Furthermore, when the number of phages or the amount of each type of bacteria reached values below 50 units per millilitre, I used a stochastic process. With a Poisson distribution, the expected number of bacteria in the next generation was calculated, using the respective mean and variance of the actual value of the variable. A 'stochastic limit' (threshold) of more than 50 units per millilitre fitted very well with the deterministic equations, meaning that there was no need to increase the threshold.

In all simulations, I used parameters with values given in Table 1.

Table 1. Symbols, values and units used in models

Symbol	Explanation	Values and/or units
X, Y	Number of bacterial cells of strain X or Y	cells · ml ⁻¹
X_p, Y_p	Number of X or Y cells bearing plasmid	cells · ml ⁻¹
P	Number of free phages	phages · ml ⁻¹
I	Number of bacteria-infected phages	cells · ml ⁻¹
ω	Rate of turnover of chemostat	h ⁻¹
α	Vegetative segregation rate	10 ⁻⁴ · h ⁻¹
C_0	Concentration of a limiting resource in the chemostat	100 µg · ml ⁻¹
s	Selective disadvantage of plasmid-bearing cells (see ψ' and ψ)	0.1
ψ	Growth rate of plasmid-free cells	1.6 · h ⁻¹
ψ_p	Growth rate of plasmid-bearing cells. It is equal to $\psi \cdot (1 - s)$	1.6 (1 - 0.1) · h ⁻¹ = 1.44 · h ⁻¹
δ	Adsorption rate of phages to cells (see equation 1)	ml · h ⁻¹
ε	Quantity of the resource needed for one cell division	10 ⁻⁶ µg for all strains
γ	Conjugational transfer parameter or 'donor ability'	ml · cell ⁻¹ · h ⁻¹
Q	Parameter used in Monod's function	5 µg · ml ⁻¹
β	Amount of phages coming out from a lysed bacterial cell (burst)	10 ⁴ phages per infected cell
τ	Latent period between infection and lysis	For MS2: 2/3 h

RESULTS

Model I: One strain only

Before considering the existence of two strains, I used equations (2) to (9) to perform several simulations but with only one strain. That is, only X and X_p cells were considered. Then, phages were added. I used different values of chemostat flow, ω , and different values of the total amount of bacteria allowed to grow in the chemostat.

The results are shown in Fig. 1A, 1B and 1C and Fig. 2. Plasmids can be maintained in the chemostat only for values above the broken line in Fig. 2 (whose analytical formula is given in equation A7; see Appendix 1). On the other hand, the MS2 phages can be maintained in the chemostat only for values above the dotted line (equation A12). Between the two quasi-straight lines one finds a 'paradise' zone where the plasmid R1 can be maintained, whereas the MS2 phages cannot. So, if phages are added, the equilibrium is temporarily destroyed over a few hours until phages are eliminated from the chemostat due to the flow (Fig. 1A) and the original equilibrium is restored.

If the parameters are such that we are in the zone between the two upper lines in Fig. 2 (the dotted and the full lines), it means that both plasmids and phages can spread in the chemostat. In this part of the parameter space, when 1000 phages are suddenly added to the chemostat where no phages are present, the equilibrium moves to another stable steady state where phages, plasmid-bearing cells and plasmid-free cells can co-exist (Fig. 1B).

Finally, in the parameter space above the irregular line in Fig. 2, both phages and plasmids can also spread. However, this time the effect of adding phages is the elimination of plasmid-bearing bacteria. Mathematically, at all points above the solid line in Fig. 2,

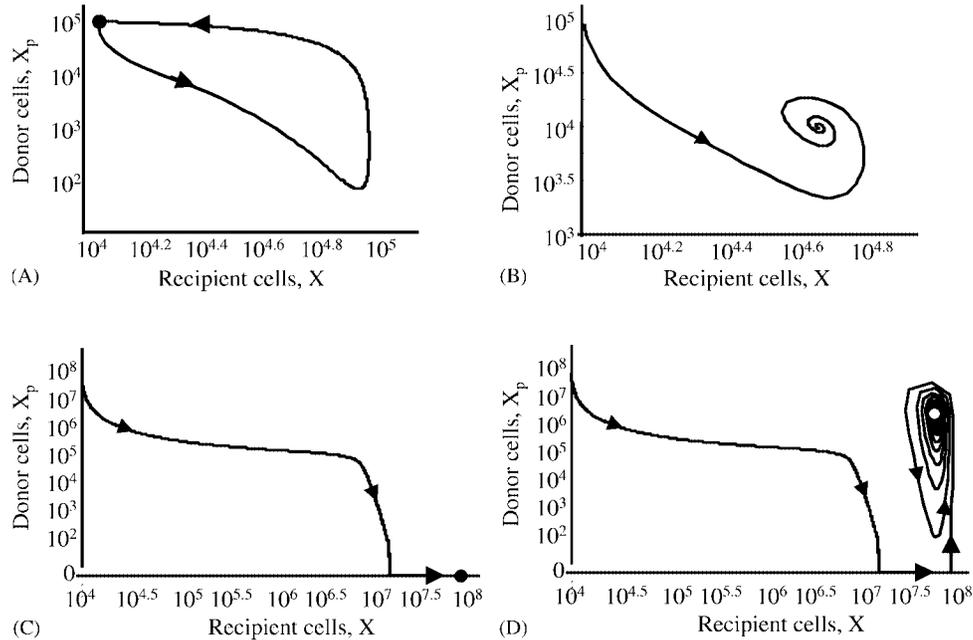


Fig. 1. The effect of introducing phages in a stable chemostat with one bacterial strain only. The system containing plasmid-bearing cells and plasmid-free cells (because there is vegetative segregation $\alpha = 10^{-4} \cdot \text{h}^{-1}$) is in a stable steady state without MS2 phages. What happens when MSPs with the characteristics of MS2 are introduced to the system? (A) Only X cells and X_p cells are present in the system; then, 10^8 MSP particles are introduced. Here the parameters are such that the system is in the *paradise zone* (see text and Fig. 2), meaning that plasmids can spread but phages cannot. The result is that the system goes back to the same state observed before the introduction of phages. (B) Only X cells and X_p cells are present in the system; then, 10^3 MSP particles are introduced. A new stable steady state is found by the system *before* any extinction occurs. In the new state, X and X_p cells co-exist with the phages. (C) Only X cells and X_p cells are present in the system; then, 10^3 phages are introduced. In contrast to (A) or (B), this time plasmid-bearing cells, X_p , are sent extinct by the phages. Only plasmid-free cells survive because MSPs cannot infect them. (D) Similar to (C), except that this time there are also bad donors (Y_p) in the system (as well as Y cells). In equilibrium, before phages were introduced, there were many Y cells that received a plasmid from X_p cells – that is, there were many Y_p cells. These bad donor cells expressing just a few sex-pili are almost uninfected by the phages. Therefore, after X_p cells go extinct, Y_p cells transfer the plasmid to X cells. Then, the system finds the stable steady state composed of plasmid-free cells, plasmid-bearing cells and phages.

there are stable steady states. However, before reaching the steady state, phages infect and eliminate all plasmid-bearing bacteria from the chemostat (Fig. 1C). Only plasmid-free cells (X cells) survive in the chemostat because they are not infected by MS2 phages (Fig. 1C).

Model I: Two strains co-exist

Now, I consider the full system (equations 2 to 9) with parameters given in Table 1 and $\omega = 0.01 \text{ h}^{-1}$. In general terms, the two strains are similar. However, different

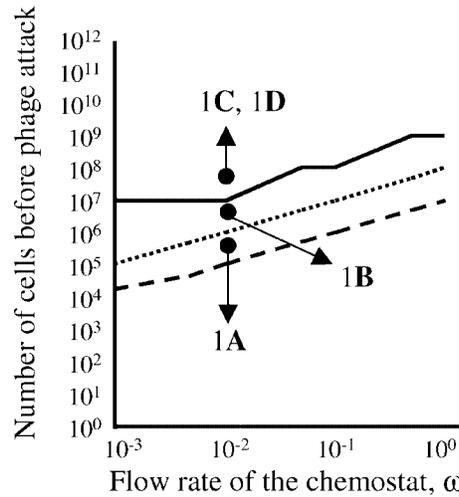


Fig. 2. Maintenance of plasmids in the chemostat after addition of MSPs. After finding the steady state when plasmid transfer rate is fast enough so that almost all bacteria harbour the plasmid (equilibrium described in Appendix 1), I checked whether plasmid-bearing bacteria are maintained in the system upon the addition of 1000 MSP particles. The dashed line corresponds to the limit under which the conjugative plasmid cannot be maintained in the system, because the transfer rate is too low (this line was obtained analytically: see equation A7). The dotted line (also obtained analytically: see equation A12) is the limit under which the phages cannot spread because the number of secondary infections of the phages is less than one. Between the dashed and dotted lines there is a *paradise zone*: here the R1 plasmid can spread but phages cannot (this corresponds to the simulation shown in Fig. 1A). Between the dotted and solid lines, we find a *'limbo' zone*: here phages can be maintained as well as plasmids and they can stably co-inhabit (this corresponds to the simulation shown in Fig. 1B). Above the solid line, the effect of adding 1000 phages in the system is the extinction of all plasmid-bearing bacterial cells, followed by the extinction of the phages due to the chemostat flow (this corresponds to the simulation shown in Fig. 1C). The solid line was obtained by performing several simulations of equations (2) to (8) with X and X_p cells only, and manipulating parameter ε to control the total amount of cells in the system ($X + X_p$). See Table 1 for parameters.

parameters related to their ability to transfer the plasmid are different: X_p cells express many more sex-pili than Y_p cells, meaning that X_p cells are much more susceptible to MSPs than Y_p cells. Accordingly, the adsorption rate to X_p cells is $\delta_x = 10^{-12} \text{ ml} \cdot \text{h}^{-1}$, the transfer rate from X_p to X cells is $\gamma_{xx} = 10^{-8} \text{ ml} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$, the transfer rate from X_p to Y cells is $\gamma_{xy} = 10^{-11} \text{ ml} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$, and the transfer rate from Y_p cells to Y and X cells is $\gamma_{yy} \equiv \gamma_{yx} = 10^{-16} \text{ ml} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$ (Dionisio *et al.*, 2002). Given that $\gamma_{yy} = 10^{-16} \text{ ml} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$, according to equation (1) $\delta_y = 10^{-20} \text{ ml} \cdot \text{h}^{-1}$, meaning that Y_p cells are hardly affected by MS2 phages. Furthermore, given such a low value of γ_{yy} , Y_p cells receive a plasmid mainly from X_p cells, not from other Y_p cells (see Dionisio *et al.*, 2002).

The simulations in Figs. 1D and 3 were done as follows. In the chemostat, the maximum number of cells of the X strain (that is $X + X_p$ cells) allowed to grow was set to approximately $10^8 \text{ cells} \cdot \text{ml}^{-1}$; accordingly, $\varepsilon_x = 10^{-6} \mu\text{g}$. Similarly, the other strain was allowed to grow and after all four types of bacterial cells (X , X_p , Y and Y_p) had stabilized, I simulated again an invasion by 1000 MSPs.

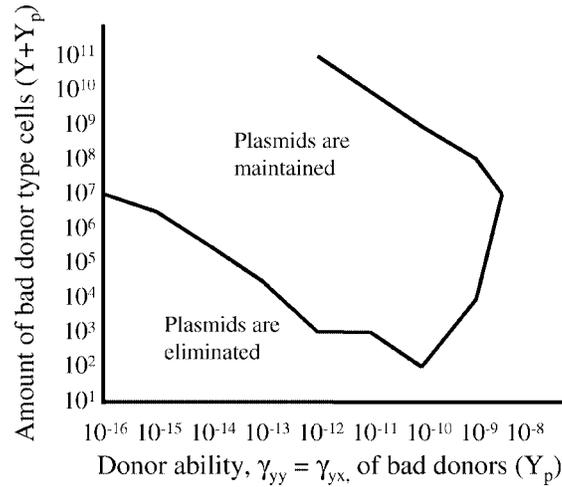


Fig. 3. Checking the number of bad donors necessary to maintain the plasmids in the chemostat. The minimum number of cells required for the population $Y + Y_p$ to represent a ‘sanctuary’ or ‘refuge’ of plasmids if $X + X_p = 10^8 \text{ cells} \cdot \text{ml}^{-1}$, with different values of transfer rate, γ_{yy} and γ_{yx} . I used the following parameters: $\omega = 0.01 \text{ h}^{-1}$, $\gamma_{xx} = 10^{-8} \text{ ml} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$, $\gamma_{xy} = 10^{-11} \text{ ml} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$ and those presented in Table 1.

Figure 1D shows the results of these simulations. As in Fig. 1C, X_p cells rapidly go extinct. However, before the extinction of phages and due to the presence of Y_p cells in the chemostat (which is hardly affected by the MS2 phage), at least some plasmids from Y_p cells are transferred to X cells. Then, a new stable steady state is found where MS2 phages, X cells, Y cells, X_p cells and Y_p cells co-exist (the small white dot in Fig. 1D).

I conducted more simulations (similar to the one in Fig. 1D) to test the minimum amount of bad donors that ensures maintenance of plasmids in the system (Fig. 3). For that, the value of ε_y was manipulated to control the maximum amount of cells of the bad donor strain ($Y + Y_p$). Then, I tested the amount of bad donors needed with different values of donor ability constants; for this purpose, simulations were performed with $\gamma_{yy} \equiv \gamma_{yx}$ equal to 10^{-16} , 10^{-15} , 10^{-14} , 10^{-13} , 10^{-12} , 10^{-11} , 10^{-10} , 10^{-9} and $10^{-8.5} \text{ ml} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$ (Fig. 3). The main results of these simulations are: (i) the minimum amount of these ‘bad donors’ (Y_p) required to maintain plasmid-bearing cells in the chemostat is never zero; (ii) the amount of ‘bad donors’ needed to ensure plasmid maintenance decreases, while donor ability increases from 10^{-16} to $10^{-10} \text{ ml} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$. It increases again when the value of donor ability is $10^{-9} \text{ ml} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$, and even more when donor ability is $10^{-8.5} \text{ ml} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$. Increasing this value no longer ensures the maintenance of plasmid-bearing cells. This is expected, as bad donors are becoming good donors themselves and therefore we are approaching a homogeneous system.

A third interesting result is the following. If the amount of bad donors is extremely high, the amount of sex-pili of bad donors is already high enough to enable phage spread. Mathematically, it means that equation (A10) in Appendix 2 is fulfilled (as well as equations A11 and A12).

Model II: Transitory derepression

In this model, I considered the existence of a single strain, but in which plasmid-bearing cells have two levels of sex-pili expression: cells that received the plasmid recently or that are direct descendents of cells that received the plasmid recently have a higher transfer rate than cells that received the plasmid many generations before [transitory derepression (model II), adapted from Lundquist and Levin (1986)]. I performed several simulations with different cell densities and manipulated the parameter m , the time that takes a recent transconjugant (with high sex-pili expression) to become a normal transconjugant with (low) repressed sex-pili expression (see Fig. 4). If all cells are constitutively derepressed, bacterial density has to be within 5×10^5 and 5×10^7 cells \cdot ml $^{-1}$ for plasmids to be maintained; if plasmids are constitutively repressed, plasmids cannot be maintained. In contrast, if there is transitory derepression, plasmids can be maintained with bacterial densities approximately between

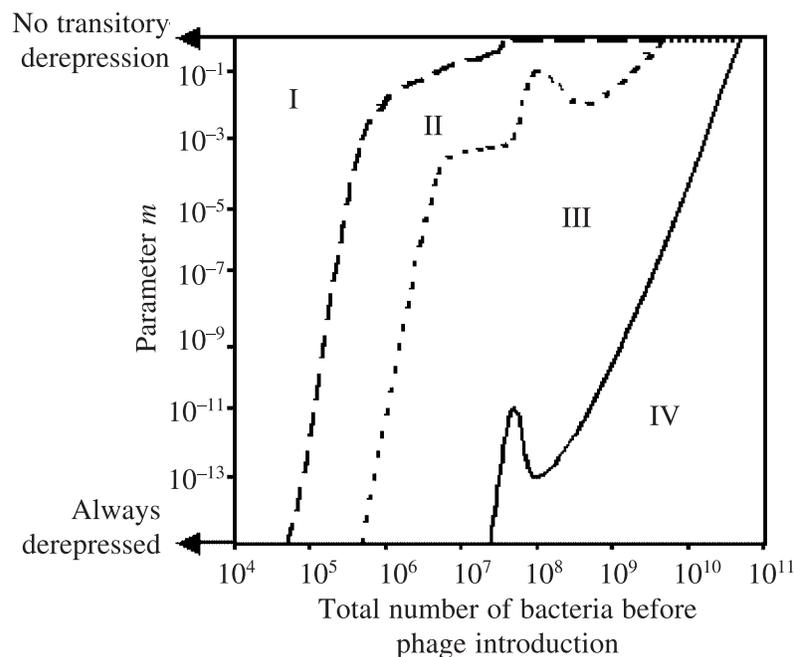


Fig. 4. The effect of transitory derepression. When a chemostat with plasmid-free plasmid-bearing bacteria – in which the plasmid is capable of transitory derepression (Lundquist and Levin, 1986) – is invaded by 1000 MS2-like MSPs, I observed three different outcomes: (I) and (IV) both plasmids and phages are eliminated from the chemostat; (II) plasmids are maintained and phages are eliminated; (III) both plasmids and phages are maintained. In the simulations I used the following parameters: $\gamma_{\text{repression}} = 10^{-11}$ ml \cdot cell $^{-1}$ \cdot h $^{-1}$, $\gamma_{\text{derepression}} = 10^{-8}$ ml \cdot cell $^{-1}$ \cdot h $^{-1}$ and $\omega = 10^{-2}$ \cdot h $^{-1}$ and those in Table 1. The parameter m is the rate at which newly formed transconjugants or close direct descendents of a newly formed transconjugant (derepressed expression of sex-pili) become repressed – low expression of sex-pili, hence a low transfer rate. If all cells are constitutively derepressed with $\gamma_{\text{derepression}} = 10^{-8}$ ml \cdot cell $^{-1}$ \cdot h $^{-1}$, plasmids are maintained if the density of bacteria is between 5×10^5 and 5×10^7 cells \cdot ml $^{-1}$; otherwise, plasmids are extinct. On the other hand, if all cells are constitutively repressed with $\gamma_{\text{repression}} = 10^{-11}$ ml \cdot cell $^{-1}$ \cdot h $^{-1}$, plasmids are maintained if the density of bacteria is higher than 1.2×10^8 cells \cdot ml $^{-1}$; otherwise, plasmids are extinct.

10^5 and 10^9 cells·ml⁻¹ if $m = 10^{-9} \cdot \text{h}^{-1}$, and with bacterial densities approximately between 10^5 and 10^{10} cells·ml⁻¹ if $m = 10^{-4} \cdot \text{h}^{-1}$ (for other values of m , see Fig. 4).

DISCUSSION

The results presented here support the conjecture that heterogeneity of donor ability increases the likelihood of conjugative plasmids to be maintained among bacterial populations. This is the case when the cost of bearing plasmids is momentarily very high (here, a sudden addition of MSPs). This conclusion was obtained through the analysis of two different systems: one in which there is bacterial diversity in sex-pili expression due to diversity of bacterial strains, and one in which only one bacterial strain is considered, though plasmids can be transiently derepressed.

Let us consider the impact of some of the simplifying hypotheses of the models. Perhaps the most non-realistic hypothesis is that bacteria are living in a chemostat. This is surely not true since, for example, most *E. coli* cells inhabit structured habitats inside mammal intestines (Whitman *et al.*, 1998). How much would the results change if modelling a structured habitat, such as a biofilm? Qualitatively, at least, there is no reason to believe that bad donor cells would cease to represent a refuge for conjugative plasmids in a structured habitat. However, the amount of bad donors needed to maintain the plasmid might be lower, as phages would have greater difficulty spreading in a structured habitat (see Schrag and Mittler, 1996). This should be studied further in future studies.

Furthermore, we assumed that all plasmid-bearing cells replicate at the same rate, no matter how intensively they express sex-pili. It would be reasonable to expect that cells with lower expression of sex-pili (bad plasmid donors) should be able to replicate faster, and this would also facilitate the maintenance of plasmid within the bad donor reservoir population (raising the line in Fig. 3).

If the conditions are such that phages can spread (see equation A12), then there are conditions for co-existence of plasmid-bearing bacteria, plasmid-free bacteria and phages. However, these equilibrium values are extremely difficult to achieve, hence the large area above the broken line in Fig. 2. Lenski (1988) had already noted that in homogeneous systems real chemostats containing phages and bacteria are more stable than expected theoretically. Therefore, Lenski concluded that there must be some heterogeneity in the system. Here, for the case of MSPs infecting bacteria expressing sex-pili, I show that heterogeneity of sex-pili expression (good donors together with bad donors, or the possibility of transient derepression) is in fact an important and sometimes fundamental factor for the stability of those bacteria in a chemostat. Furthermore, the help of bad donors is important *solely* under non-equilibrium conditions (e.g. sudden invasion of MS2 phages), *not* in stable conditions. That is, the mere presence of bad donors in the chemostat does not generate a new stable steady state. The effect of bad donors is to increase the size of the basin of attraction of that steady state. Moreover, even if equilibrium is reached with the help of bad donors (e.g. Fig. 1D), the equilibrium will be maintained *a posteriori* even if bad donors are eliminated from the chemostat.

Bacterial cells as habitats for plasmids

Plasmids are totally dependent on their hosts: unlike phages or viruses in general, plasmids cannot 'survive' outside living cells, hence they can transfer horizontally only if they are

already inside a cell. Therefore, one can consider that plasmids are living in different 'habitats', those 'habitats' being different cells of bacterial strains or species. Following this metaphor, and from the point of view of conjugative plasmids, bacterial cells in which sex-pili are derepressed (hence a high transfer rate) are high-quality habitats, whereas cells in which sex-pili are repressed (hence a low transfer rate) are low-quality habitats. Indeed, defined in this way these two 'habitats' are in the conditions proposed by Jansen and Yoshimura (1998) of a population able to persist in an environment consisting of sink habitats only.

Sink habitats, by definition, are habitats in which populations cannot survive when they are isolated from other populations (Pulliam, 1988; Dias, 1996). In their theoretical model, Jansen and Yoshimura (1998) 'suppose that an individual distributes its offspring over two types of habitat with different qualities. The probability of offspring survival differs between the two habitats . . . One habitat . . . is highly productive but suffers from occasional catastrophes in which only very few offspring survive . . . The other habitat is constant in quality but the quality is low so that a population that uses this habitat only is doomed to extinction'.

The models presented here fit the conditions described by Jansen and Yoshimura (1998). Indeed, the population of bacteria where plasmids have difficulty in expressing sex-pili, Y_p , corresponds (from the plasmid point of view) to the constant habitat of low quality (doomed to extinction because the plasmid transfer rate does not compensate for the chemostat flow, segregation and fitness costs of harbouring the plasmid). The population of bacteria in which plasmids can easily express sex-pili for horizontal transfer, X_p , is a habitat of very high quality but *which suffers from occasional* attacks by MSPs (catastrophes). Therefore, the models presented in this paper describe plasmids inhabiting a patchy environment composed of two sink habitats only – X_p and Y_p cell types – and plasmids need both types of habitats to be maintained simultaneously in the chemostat. Following this allegory, migration between the two sink habitats corresponds to plasmid transfer between cells belonging to two different strains.

What happens if a system consisting of both good and bad donors and without transitory derepression loses one of the strains? If MSPs are not in the system the plasmid is prone to go extinct: if good donors are eliminated for some reason, then plasmids are going to disappear because they cannot spread in bad donors; if bad donors disappear, and given that the probability of MSPs to appear is very high, the extinction of plasmids is also to be expected.

However, bad donors are 'useful' for plasmid maintenance only while MSPs are not present in the system; indeed, if phages, plasmid-free and plasmid-bearing bacteria of both strains are in equilibrium, the eventual elimination of bad donors would not place the other strain in danger. Mathematically, this is because the system is already in the basin of attraction. The biological (and more intuitive) reason is that, even if a new type of MSP suddenly arises, it will not cause a strong instability in the system because the number of good donors bearing the plasmid was already being kept low due to co-existence with the first type of MSP.

In the transitory derepression model, we see the following composition of cells expressing high versus low numbers of sex-pili: initially, when most cells are free of plasmids, the transfer rate is enhanced – transitory derepression. In a few minutes, some transconjugants become repressed, hence representing a poor habitat (from the perspective of plasmid transfer) but safe from infection by MSPs. When the frequency of plasmid-bearing cells

increases, the advantage of producing conjugative pili declines and the final result is a system in which most plasmid-bearing cells hardly express sex-pili. So, even if some MSPs infected a few cells at the beginning of plasmid spread, eventually MSPs go extinct given that sex-pili expression becomes repressed. In other words, transitory derepression helps to generate a patchy environment for conjugative plasmids, hence contributing to the maintenance of conjugative plasmids during initial plasmid spread and upon MSP emergence; later, most cells repress sex-pili expression and MSPs cannot invade.

An evolutionary conjecture

Following Lundquist and Levin (1986), if horizontal transfer is the main force maintaining conjugative plasmids, then repression of conjugative pili synthesis is paradoxical from the evolutionary biology standpoint. However, the paradox disappears when we take into consideration the following three facts already outlined in this paper: (i) cells harbouring plasmids repressed for conjugative pili synthesis divide faster than cells containing similar plasmids though constitutively derepressing conjugative pili synthesis (Levin, 1980); (ii) cells bearing plasmids that are repressed for conjugative pili synthesis are less susceptible to MSPs than cells containing plasmids permanently derepressed for conjugative pili synthesis; (iii) if there is an intrinsic disadvantage in expressing sex-pili [e.g. a cost related to the synthesis of sex-pili (see Levin *et al.*, 1979)], the advantage of producing these pili declines with the increase of the frequency of plasmid-bearing cells. Once the plasmid is common enough, the intrinsic disadvantage overcomes the infectious transfer advantage. Therefore, repression of sex-pili synthesis can be regarded as a compromise between the advantages and costs of infectious transmission (I thank the editor for calling my attention to this point). Lundquist and Levin (1986) suggest that, 'as a consequence of retransfer by transitorily derepressed cells, this compromise need not preclude the maintenance of these replicons by infectious transfer alone'. In their study, Lundquist and Levin (1986) found that the R1 plasmid and three of five plasmids isolated from faecal samples of humans failed to exhibit transitory derepression of sex-pili synthesis. These plasmids may have lost this phenotype (e.g. the R1 plasmid was isolated 20 years before that study and may have lost the ability to transitorily derepress conjugative pili synthesis). I speculate that another possibility is that these plasmids have relied for their maintenance on bacterial host diversity. This suggestion requires an explanation.

It has been shown that the donor ability of the plasmid R1 spans at least six orders of magnitude among *E. coli* strains both in well-mixed liquid nutrients and in a surface (Gordon, 1992; Dionisio *et al.*, 2002). In contrast to this diversity, if the R1 plasmid has its repressor genes mutated (hence the plasmid is derepressed, and constitutively expresses sex-pili), then there is no diversity of the donor ability phenotype (Dionisio *et al.*, 2002). This suggests that repression of conjugative pili is an adaptive phenotype to cope with differences among possible bacterial hosts. Accordingly, it is interesting to note here that the diversity of transfer ability of the R1 plasmid vanishes if one previously cures the strains to eliminate putative resident plasmids, suggesting that other plasmids may be responsible for the diversity in sex-pili synthesis (Dionisio *et al.*, 2002). Does this mean that there is a benefit in designing a repressor susceptible to variations among bacteria in terms of bet hedging (Cohen, 1966)? I leave this unanswered question for future research.

In conclusion, the present study suggests that, both as a consequence of retransfer by transitorily derepressed cells and of diversity in repression levels among different hosts,

conjugative plasmids can successfully spread among bacterial hosts with a low risk of being eliminated by MSPs.

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APPENDIX 1

Consider the system defined by equations (4), (6) and (8) and assume that the chemostat just contains X and X_p cells and nutrients with concentration C_x (so, there are no phages or any other type of cells or nutrients, $P(t) = I(t) = Y(t) = Y_p(t) = C_x(t) = 0$). Such a system has three steady states, two of them unstable. An unstable steady state is the one in which only nutrients are present, ($\hat{C}(t) = C_0$), while bacteria are not there ($\hat{X}(t) = 0$, $\hat{X}_p = 0$). The ^ sign indicates the respective value of equilibrium steady states. Another possible steady state is the one in which the chemostat only harbours nutrients and plasmid-free bacteria:

$$\hat{X} = \frac{C_0(\psi - \omega) - Q\omega}{\varepsilon(\psi - \omega)} \quad (\text{A1})$$

$$\hat{X}_p = 0 \quad (\text{A2})$$

$$\hat{C} = \frac{Q\omega}{(\psi - \omega)} \quad (\text{A3})$$

There is a third steady state solution. If we assume that plasmid segregation is very rare, $\alpha \approx 0$, this steady state is

$$\hat{X} = 0 \tag{A4}$$

$$\hat{X}_p = \frac{C_0(\psi_p - \omega) - Q\omega}{\varepsilon(\psi_p - \omega)} \tag{A5}$$

$$\hat{C} = \frac{Q\omega}{(\psi_p - \omega)} \tag{A6}$$

The last two steady states (equations A1–A3 or A4–A6) cannot be stable simultaneously. Either all bacteria are plasmid-free, or assuming there is no segregation ($\alpha = 0$), all bacteria harbour the plasmid. In the presence of bacteria and nutrients, equations (A1)–(A3) represent a stable steady state if and only if the transfer rate of the plasmid is not very fast, that is if $\gamma_{xx} < \frac{s\omega}{\hat{X}}$, where \hat{X} is given by equation (A1) and represents the total number of bacterial cells in the system in the absence of plasmids (Stewart and Levin, 1977). Substituting \hat{X} in equation (A1) we get:

$$\gamma_{xx} < \frac{(\psi - \omega)s\omega\varepsilon}{C_0(\psi - \omega) - Q\omega} \tag{A7}$$

Otherwise, all bacteria harbour the conjugative plasmid and equations (A4)–(A6) describe the equilibrium steady state. If $\alpha > 0$, some of the X_p cells become plasmid-free cells, so in equation (A4) we get $\hat{X} \approx \frac{\alpha}{\gamma_{xx}}$ and the concentration of nutrients in the chemostat would have a very similar value to that given by equation (A6). For $\alpha > 0$, condition (A7) becomes

$$\gamma_{xx} < \frac{(\psi - \omega)(s\omega + \alpha)\varepsilon}{C_0(\psi - \omega) - Q\omega} \tag{A8}$$

The parameter γ_{xx} is a measure of fertility, easily *translated* to the R_0 parameter commonly found in studies with epidemiological models. R_0 is the number of secondary infected hosts expected from a primary case in a completely susceptible population (\hat{X} of equation A1), and relates to γ_{xx} as

$$R_{0 \text{ (plasmid)}} = \frac{\gamma_{xx}}{\frac{(\psi - \omega)(s\omega + \alpha)\varepsilon}{C_0(\psi - \omega) - Q\omega}} = \frac{\gamma_{xx}(C_0(\psi - \omega) - Q\omega)}{(\psi - \omega)(s\omega + \alpha)\varepsilon} \tag{A9}$$

Therefore, equations (A8) and (A9) mean that plasmids spread if and only if $R_{0 \text{ (plasmid)}} > 1$.

APPENDIX 2

If there is no plasmid segregation ($\alpha = 0$), but the chemostat contains MSPs, then the co-existence of plasmid-free and plasmid-bearing bacteria becomes possible. This happens because MSPs specifically infect plasmid-bearing cells. Therefore, MSPs set the quantity of these cells expressing sex-pili below their ‘natural carrying capacity’ (given in equation A5). This leaves some nutrients available to plasmid-free cells.

Initially, if there are no MSPs, and equations (A4)–(A6) describe the steady state of the system, what is the condition for MSPs to be able to invade the bacterial population? The condition can be

obtained using equations (2), (3) – Y and Y_p are not considered here – and (A5): $\delta_x > \frac{\omega e^{\omega\tau}}{\hat{X}_p(\beta - 1)}$, where \hat{X}_p is the number of plasmid-bearing bacteria *just before* phages start spreading (equation A5). That is,

$$\delta_x > \frac{1}{\beta - 1} \frac{\omega \varepsilon (\psi' - \omega) e^{\omega\tau}}{C_0(\psi' - \omega) - Q\omega} \quad (\text{A10})$$

The phage spreads if this condition is verified. In other words, phages spread if the number of secondary infected cells expected from a primary case in a completely susceptible population, R_0 (phage), is greater than one. According to this, we can readily calculate R_0 (phage) from the last equation:

$$R_0 \text{ (phage)} = \frac{(\beta - 1) (C_0 (\psi' - \omega) - Q\omega)}{\delta_x \omega \varepsilon (\psi' - \omega) e^{\omega\tau}} \quad (\text{A11})$$

Furthermore, considering that $\gamma_{xx} = k\delta_x$, equation (A10) becomes:

$$\gamma_{xx} > \frac{k}{\beta - 1} \frac{\omega \varepsilon (\psi' - \omega) e^{\omega\tau}}{C_0(\psi' - \omega) - Q\omega} \quad (\text{A12})$$

Consider that the expression that limits γ_{xx} in equation (A12) (let us call it $\gamma_{xx(\text{A12})}$) is higher than γ_{xx} of equation (A7), $\gamma_{xx(\text{A7})}$. Then, there is an interval of γ_{xx} where conjugative plasmids can spread, though MSPs cannot. Such a ‘paradise’ zone for plasmids exists if:

$$\gamma_{xx(\text{A12})} > \gamma_{xx(\text{A7})} \quad (\text{A13})$$

That is, if:

$$\frac{e^{\omega\tau} k(\psi(1 - s) - \omega)}{\beta - 1} > (\psi - \omega)s \quad (\text{A14})$$

As already explained, in this paper we consider experimental values of parameters k and β for the phage MS2. They are equal to $10^4 \cdot \text{cell}^{-1}$ and 10^4 phages $\cdot \text{cell}^{-1}$, respectively, and equation (A14) becomes:

$$e^{\omega\tau} (\psi(1 - s) - \omega) > (\psi - \omega)s \quad (\text{A15})$$

where we assume that $\beta \approx \beta - 1$.

It can be seen from equation (A15) that such a ‘paradise’ zone can indeed exist. For example, assuming that the growth rate is $\psi = 1.6 \cdot \text{h}^{-1}$ in rich medium, that the fitness cost for harbouring a plasmid is $s = 0.1$, that the flow of the chemostat is $\omega = 0.01 \cdot \text{h}^{-1}$ and that the time lag between phage infection and cell lysis is $\tau = 0.01$ h, we get the values in each side of equation (A15): $1.441 > 0.159$, while if $\omega = 0.1 \cdot \text{h}^{-1}$, we get $1.353 > 0.15$.

When condition (A10) is fulfilled, what is the equilibrium steady state of equations (2), (3), (4), (6) and (8) when considering only one type of cells (that is, $Y = Y_p = 0$)? Noting that the concentration of nutrients available is usually much less than C_0 , we can consider that $C_0 - C \approx C_0$ and that $Q + C \approx Q$. Moreover, considering that the effect of plasmid segregation (given by α) is negligible compared with the effect of the phages, we get the following approximated solution:

$$\hat{X}_p = \frac{\omega}{\delta_x \beta} \quad (\text{A16})$$

$$\hat{C} = \frac{(\gamma_{xx} \hat{X}_p + \omega) \underline{Q}}{\psi} \quad (\text{A17})$$

$$\hat{X} = \frac{\omega C_0 \underline{Q}}{\varepsilon \psi} - (1-s) \hat{C} \hat{X}_p \quad (\text{A18})$$

$$\hat{P} = \frac{\psi' \hat{C} \underline{Q} + \hat{X} \gamma_{xx} - \omega}{\delta} \quad (\text{A19})$$

$$\hat{I} = \frac{\delta \hat{X}_p \hat{P} (1 - e^{-\omega \tau})}{\omega} \quad (\text{A20})$$

in which \hat{X}_p , \hat{C} , \hat{X} and \hat{P} that appear in these equations are given in equations (A16), (A17), (A18) and (A19) respectively.

