

The Persistence of Parasitic Plasmids

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ABSTRACT

The conditions under which plasmids are predicted to persist remain controversial. Here, we reevaluate the ordinary differential equations used previously to model plasmid persistence and conclude that the parameter space required for maintenance is far less stringent than has been supposed. Strikingly, our model demonstrates that purely parasitic plasmids may persist, even in the absence of heterogeneity in the host population, and that this persistence is expressed by oscillations or damped oscillations between the plasmid-bearing and the plasmid-free class.

A key concept from the genomics era is the partition of the bacterial genome into “core” and “accessory” elements. The former includes all those genes responsible for essential housekeeping functions of the cell and defines the “essence” of a given taxonomic unit by excluding any genes not present in all strains. The presence of accessory elements, in contrast, is highly variable between strains and although the functions of these genes tend to be less clear they are generally thought to be responsible for microadaptations at the subspecies level. Although primarily conceptual, this partition can also be physical, with core genes being confined to the bacterial chromosome while accessory genes are found on semiautonomous replicons such as plasmids or (as in the case of the Burkholderia, Rhizobium, or Vibrio genera) secondary chromosomes that are likely to have evolved from megaplasmids. Given certain environmental conditions, plasmids can provide many different types of selective advantage, including antibiotic resistance, resistance to pollutants or UV, and biofilm formation (CHIGO 2001; DIONISIO 2005).

Plasmids thus make a major contribution to the accessory gene pool, but they are also considered to impose a fitness cost related to plasmid carriage and the time and resources required to replicate extra DNA. The precise magnitude and consistency of the fitness cost imposed by plasmids are currently a matter of debate. DIONISIO *et al.* (2005) have recently suggested that this cost ameliorates over evolutionary time and that initially costly plasmids may eventually confer a selective advantage. More recently the model of GELDER *et al.* (2004) suggested that the fitness cost may change somewhat erratically over time due to stochastic plasmid and

chromosomal mutations. DIONISIO (2005) has discussed a different kind of fitness cost, where the synthesis of sex pili leaves the cell vulnerable to male-specific phages. Dionisio proposes that heterogeneity in the expression of sex pili can facilitate the maintenance of plasmid (and phage) within the bacterial population.

In addition to conferring a potential fitness cost, plasmids may be lost stochastically during bacterial reproduction by failing to segregate into one of the daughter cells during binary fission. PONCIANO *et al.* (2006) note that in some strains this stochastic segregation loss may be an important effect even in plasmids of low cost. A high copy number of plasmids per cell can dramatically reduce the risk of stochastic plasmid loss; for example, pBR322 has a copy number of ~ 21 , meaning that in theory only 1 cell in 1 million would lose all plasmids by stochastic loss (SORENSEN *et al.* 2005). However, a trade-off exists as an increase in copy number imposes an increased cost to the cell (PAULSSON 2002). Larger plasmids tend to have a lower copy number, but these often contain genetic systems (*e.g.*, the stable partitioning, or *par*, gene) that act to reduce the probability of daughter cells not receiving a plasmid. The limited evidence available from *in situ* studies suggests that local adaptation to a microniche will also lead to increased plasmid–host stability (SORENSEN *et al.* 2005).

Despite these caveats, a combination of fitness cost and stochastic loss should, over time, act to remove plasmids from the bacterial population. Much attention has therefore been focused on the nature of the opposing forces that act to maintain plasmids, and two such forces are widely considered important: the rate of infectious (horizontal) transfer between bacteria (in this case the dynamics are similar to the trade-offs in parasite–host dynamics) and the strength of the selective advantage conferred by plasmid genes. There are reasons to doubt the ability of either of these forces to

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maintain plasmid-bearing cells indefinitely. First, the selective advantage conferred by plasmids is likely to be evident only under very specific environmental conditions (such as in the presence of an antibiotic), and it is possible that the host may circumvent the cost of plasmid maintenance through the transfer of adaptive genes from the plasmid to the bacterial chromosome. Furthermore, in many cases the precise selective advantage conferred by some plasmids may be negligible or nonexistent, and such plasmids may be acting purely parasitically. Second, *in vitro* estimates of transfer rates, population density, segregation rates, and fitness costs have suggested that infectious transfer *per se* is insufficient for plasmid maintenance (GERDES *et al.* 1986; MONGOLD 1992; PAULSSON and EHRENBERG 1998; BERGSTROM *et al.* 2000; PAULSSON 2002). DIONISIO *et al.* (2005) noted that heterogeneity in transfer rates, more specifically the presence of a small number of “superdonors,” can greatly enhance plasmid spread within a population. Furthermore, the studies of BERGSTROM *et al.* (2000) are based on transfer rates in liquid culture and do not take into account that natural bacterial communities commonly exist as biofilms, where cell densities and hence rates of infectious transfer are expected to be much higher (SORENSEN *et al.* 2005). There is some recent strong evidence in support of transfer rates being sufficient to support plasmid populations on bacterial mats (BAHL *et al.* 2007) and on rat endothelial cells (BAHL *et al.* 2006). Indeed the ability to form biofilm may be conferred by the plasmid itself, thus providing a means by which the plasmid may be maintained though high rates of transfer in addition to allowing the bacteria to survive harsh environments (IMRAN *et al.* 2005).

To understand the precise conditions under which plasmids are maintained in bacterial populations, numerous mathematical models and experimental studies have been presented in recent years (MONGOLD 1992; PAULSSON and EHRENBERG 1998; BERGSTROM *et al.* 2000; BERG and KURLAND 2002; PAULSSON 2002; TURNER 2004; DIONISIO 2005; WILLMS *et al.* 2006). Notable among these was the report by BERGSTROM *et al.* (2000, p. 1506) who stated that “plasmids cannot persist simply by bearing genes that are beneficial to their hosts” and instead invoked the ability of plasmids to “hitchhike” on the adaptive bacterial mutants responsible for selective sweeps or to transfer selective genes between populations adapted to different niches. Given the uncertainties concerning fitness cost, segregation rates, transfer rates, and selective advantage we were motivated to re-evaluate the model of BERGSTROM *et al.* (2000) to clarify the parameter space in which plasmids are predicted to be lost from bacterial populations. Surprisingly, by analyzing the ordinary differential equations used by these authors we reached a fundamentally different conclusion: that plasmid-bearing cells may in fact persist even in homogenous bacterial populations by undergoing oscillations with the plasmid-free class. This is predicted

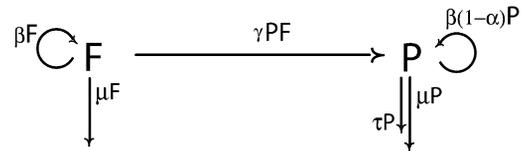


FIGURE 1.—The simple model describing horizontal gene transfer by conjugation. Plasmid-free cells (F) become plasmid-bearing (P) by direct contact. Conjugation is assumed to occur as an infective process following the law of mass action at a rate γ . Vertical reproduction and death rates are β and μ , respectively, whereas α denotes the fitness cost related to plasmid carriage. Losses due to vegetative segregation decrease the P class at a rate τ .

to be the case even when plasmids confer no selective advantage to the bacteria and may even confer a significant cost. We note that the requirement for alternative explanations for plasmid maintenance, including selective sweeps, or infectious transfer between “ecotypes” is diminished and argue that plasmid maintenance does not require a heterogeneous selective landscape. Recent evidence concerning fitness costs and transfer rates in natural communities lends weight to this suggestion.

MATHEMATICAL METHODS AND RESULTS

Novel analytical and numerical results based on a previous model: The model presented in the article by BERGSTROM *et al.* (2000) describes the plasmid infection and gene transfer from plasmid-bearing to plasmid-free bacterial cells (Figure 1). Initially, no growth-rate benefits for the cell host carrying the plasmid are taken into account.

A system of two ordinary differential equations describes the transmission of plasmids from plasmid-bearing (P) cells to plasmid-free (F) cells occurring at an infection rate γ . Both P and F have the same death rate μ but different growth rates, β for F and $\beta(1 - \alpha)$ for P , since the carriage of the plasmids imposes a cost, α , on the growth rate of the bacteria. Stochastic losses due to vegetative segregation decrease the P class at a rate τ [system (1)]:

$$\begin{aligned}\frac{dF}{dt} &= \beta F - \gamma PF - \mu F \\ \frac{dP}{dt} &= \beta(1 - \alpha)P + \gamma PF - \tau P - \mu P.\end{aligned}\quad (1)$$

BERGSTROM *et al.* (2000) assumed that, in addition to satisfying the equations of system (1), F and P also satisfy the inequality

$$\frac{P'(t)}{P(t)} < \frac{F'(t)}{F(t)}.\quad (2)$$

(Stewart and Levin’s criterion)

From this, they concluded that plasmids cannot persist in the absence of selection favoring the bacteria that carry them. Here, we show that solutions of system (1) do not in general satisfy (2) for all t and that in some cases plasmids may persist. We analyze system (1) and expansions of it by using analytical and numerical methods.

In BERGSTROM *et al.* (2000), the coefficients α , β , γ , τ , and μ were taken to be general functions of t . We first consider the case where these coefficients are constant. We assume that $\beta(1 - \alpha) < \tau + \mu$. If this were not the case, such that $\beta(1 - \alpha) > \tau + \mu$, the birth rates of the P cells would overwhelm their death rate and $P \rightarrow \infty$, which is unrealistic. We also assume that $\beta > \mu$; *i.e.*, the growth rate of the plasmid-free cells is greater than their death rate. If $\beta < \mu$ then $(P, F) \rightarrow (0, 0)$ and the population dies out. Then, for intermediate birth rate, $\mu < \beta < (\tau + \mu)/(1 - \alpha)$, the nontrivial steady state of system (1), corresponding to the coexistence equilibrium of plasmid-bearing and plasmid-free cells, is in the positive quadrant (so $F^* > 0$, $P^* > 0$, meaning that the equilibrium is realistic):

$$(F^*, P^*) = \left(\frac{\tau + \mu - \beta(1 - \alpha)}{\gamma}, \frac{\beta - \mu}{\gamma} \right).$$

Then the above trivial steady state of system (1), where both populations die out, is a saddle point and the Jacobian matrix at the coexistence state has two imaginary eigenvalues (see the APPENDIX). Additionally, taking into account that system (1) is conservative (the equations of the system can be integrated once by separable variables giving the associated “conservation law”), the coexistence equilibrium is a center and the solutions of the equation presenting the phase plane, $f(F, P) = A$, where A is a constant, are closed curves (similarly to the classical Lotka–Volterra prey–predator model (VOLTERRA 1926; LOTKA 1932)). In other words, the plasmid-bearing cells (P) can coexist with the plasmid-free cells (F) and oscillate around a neutrally stable steady state, (F^*, P^*) , the center of the closed curves (Figure 2).

This shows that any nonconstant solution of system (1) with positive initial conditions is periodic and is enough to prove that Stewart and Levin’s criterion (2) does not always hold. In each period, there is a point with $F'(t) = 0$ and $P'(t) > 0$, so that $P'(t)/P(t) \not\leq F'(t)/F(t)$.

We can now predict that plasmids can persist in a sense of surviving under continuing oscillations between the plasmid-free and the plasmid-bearing cells. Under the conditions where $\mu < \beta < (\tau + \mu)/(1 - \alpha)$, the population classes are not driven to extinction and the system is driven to oscillations and coexistence. This is true even when there is a high cost to plasmid maintenance. This completes the proof that if (1) holds with constant coefficients then (2) cannot hold for all t . The case where the coefficients are not constant is more difficult to analyze. However, let us assume that at

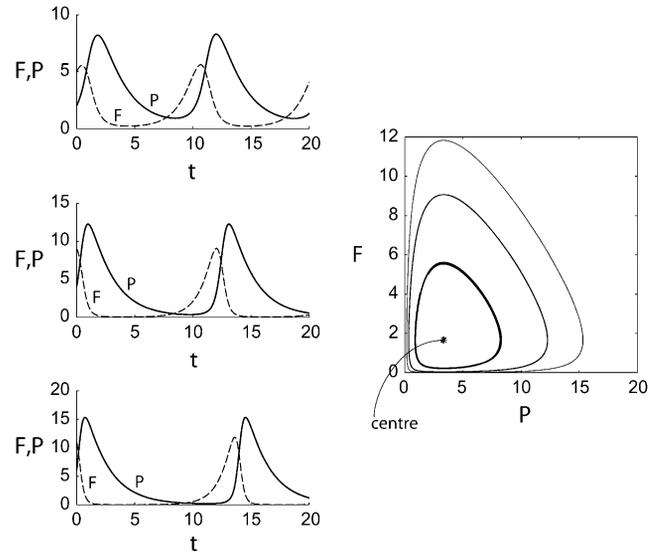


FIGURE 2.—The behavior of plasmid-free and plasmid-bearing solutions based on system (1) for three different initial conditions $[(F_0, P_0) = (5, 2), (9, 4), (11, 6)]$. It is clear that plasmid-free (F) in correspondence with plasmid-bearing (P) solutions give closed periodic curves around the center. The parameter values for the model of system (1) used here are $\beta = 1.2$, $u = 0.2$, $\tau = 0.03$, $\alpha = 0.9$, and $\gamma = 0.3$. Other parameter values give similar behavior as long as $\mu < \beta < (\tau + \mu)/(1 - \alpha)$.

time t_0 , then $\mu(t_0) < \beta(t_0) < (\tau(t_0) + \mu(t_0))/(1 - \alpha(t_0))$ [if there is no such time then either $P \rightarrow \infty$ or $(P, F) \rightarrow (0, 0)$, as before]. Choose initial conditions at t_0 with $F(t_0) = (\tau(t_0) + \mu(t_0) - \beta(t_0)(1 - \alpha(t_0)))/\gamma(t_0) + \epsilon$ and $P(t_0) = (\beta(t_0) - \mu(t_0))/\gamma(t_0)$, for some $\epsilon > 0$. Then Stewart and Levin’s criterion (2) is not satisfied at t_0 and therefore does not hold for all t . This does not rule out the possibility that Stewart and Levin’s criterion (2) may hold for all t in some cases and with some initial conditions; the oscillatory nature of Equation 1 mitigates against this, and we suggest that it must be verified in any particular case before any conclusions are drawn from it.

Moving further, we examined the dynamics of the model when plasmid-bearing cells have an advantage in growth rate (*e.g.*, when plasmids code for advantageous genes) compared with the plasmid-free cells (APPENDIX). Similarly to the previous situation, results based on the same analytical and numerical analysis indicate oscillatory behavior and coexistence of both plasmid-free and plasmid-bearing cells. Following the lead of BERGSTROM *et al.* (2000), we then incorporated the possibility of adaptive gene transfer, from the plasmid to the host chromosome. This extension gives rise to another population class, the C class, describing the cells able to incorporate into their chromosome the novel gene sequence that was transferred by the plasmid (Figure 3).

The C class escapes the cost of plasmid carriage conferred on the P class but retains the growth advantage of

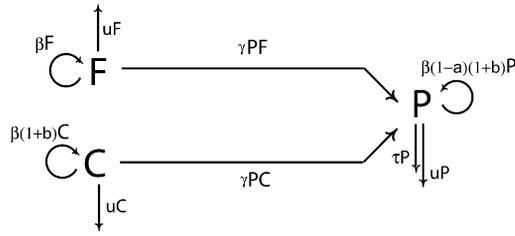


FIGURE 3.—The expanded model including the chromosomal cells, which incorporate the plasmid genes into their chromosome. The latter do not suffer from the carriage of plasmids and do have the benefit (in their growth rate) of the advantageous genes transferred by the plasmids.

the F class. It is clear and is shown mathematically that plasmid-free cells are outcompeted by chromosomal cells, so that $F(t)$ becomes negligible compared to $C(t)$ as $t \rightarrow \infty$ and therefore we can investigate only the behavior of the P and C classes (APPENDIX). The dynamics are consequently similar to the previous ones but now with oscillations between the P and the C class (Figure 4) and closed curves around the neutrally stable steady state.

This analysis demonstrates that the transfer of selective genes to the chromosome will result in the extinction of the plasmid-free F class, which has not acquired the gene, but not to the extinction of the plasmid-bearing P cells.

Expanding the model to explicitly consider the action of competition for resources: BERGSTROM *et al.* (2000) considered resource competition implicitly through time-dependent coefficient fractions. Here, we propose a generalized model [system (3)] that considers competition for resources explicitly. We assume that the growth rate of any single class is limited by the carrying capacity of the environment, k , modeled according to the standard logistic law. Again using analytical and numerical results we note the conditions required for plasmid persistence and invasion:

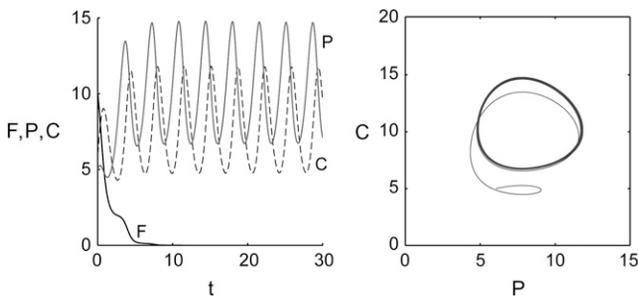


FIGURE 4.—Oscillatory behavior and competition between plasmid-bearing (P) and chromosomal cells (C). Plasmid-free cells (F) die out and plasmids remain present at the population in the P class. Parameter values used are $\beta = 0.8$, $\gamma = 0.2$, $\alpha = 0.4$, $b = 1$, $\mu = 0.05$, and $\tau = 2.95$.

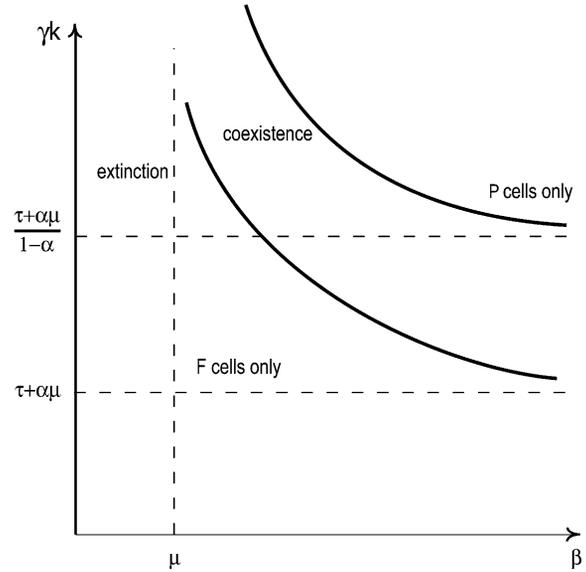


FIGURE 5.—Population behavior of system (3) for possible conditions of cell growth rate (β) and plasmid transfer rate at carrying capacity (γk).

$$\begin{aligned} \frac{dF}{dt} &= \left[\beta \left(1 - \frac{F+P}{k} \right) - \gamma P - \mu \right] F \\ \frac{dP}{dt} &= \left[\beta(1-\alpha)(1+b) \left(1 - \frac{F+P}{k} \right) + \gamma F - \tau - \mu \right] P. \end{aligned} \tag{3}$$

In the system described above, plasmid-free (F) and plasmid-bearing (P) classes are competing equally under the same environmental carrying capacity k . The behavior of this modified system is sketched in Figures 5 and 6. The damped oscillations show that Stewart and Levin’s criterion (2) does not hold for all t with these parameter values. The population goes extinct if the birth rate is less than the death rate, $\beta < \mu$, as expected. If $\beta > \mu$, then it is possible to get F cells only, coexistence of F and P cells, or P cells only, depending on the values of β and γk . The parameter combination γk may be thought of as the plasmid transfer rate at carrying capacity and it is intuitively clear that large values of γk will tend to favor P cells. Large values of β are necessary to allow P cells to persist in competition with F cells because $\beta(1-\alpha)$ has to dominate $\tau + \mu$ (rather than simply β dominating μ).

This resource-limited system may be extended in the same way as the unlimited system (1) to include C cells. In that case, it can be shown as for the unlimited system that the F class dies out as time goes to infinity. So, in effect, only plasmid-bearing (P) and chromosomal (C) cells compete with each other. The outcome depends on the values of β and γk in a similar way to the system with no F cells.

Finally, we considered the consequences of the introduction of an adaptive mutation to the plasmid-free

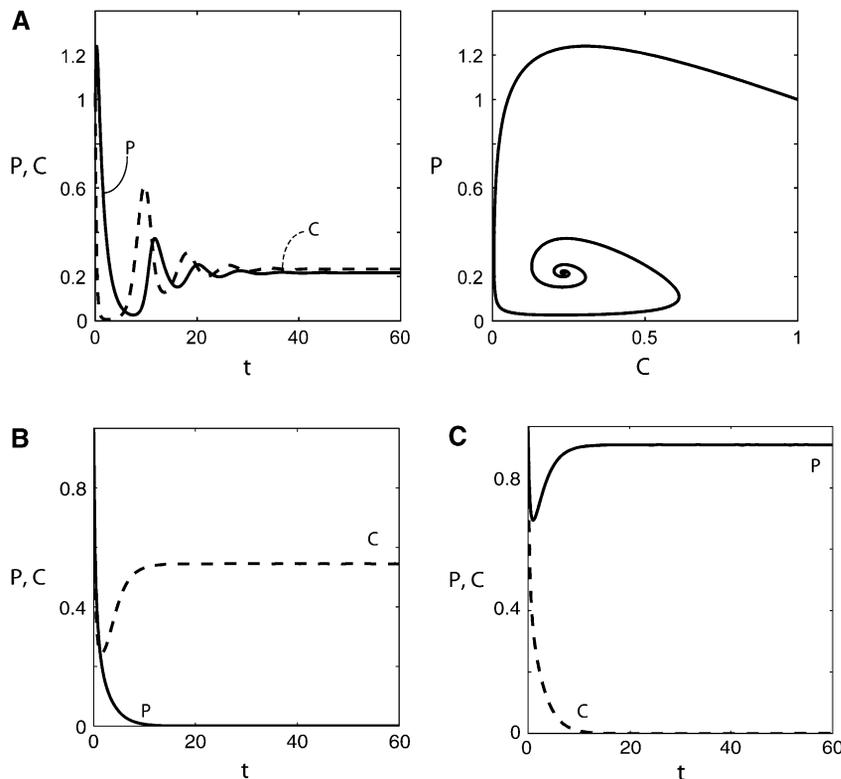


FIGURE 6.—Numerical results for the various equilibria of system (3). (A) Damped oscillations between plasmid-bearing and chromosomal cells leading to the coexistence steady state of system (3). Parameter values are $\alpha = 0.8$, $\beta = 1$, $b = 0.2$, $\gamma = 3$, $\mu = 0.1$, $\tau = 0.8$, and $k = 1$. Initial conditions: $P_0 = C_0 = 1$. (B) Numerics of the plasmid-bearing equilibrium of system (3). Parameter values are $\alpha = 0.8$, $\beta = 2$, $b = 6$, $\gamma = 3$, $\mu = 2$, $\tau = 0.1$, and $k = 1$. Initial conditions: $P_0 = C_0 = 1$. (C) Numerics of the chromosomal cells equilibrium of system (3). Parameter values are $\alpha = 0.08$, $\beta = 2$, $b = 0.1$, $\gamma = 0.5$, $\mu = 0.1$, $\tau = 0.01$, and $k = 1$. Initial conditions: $P_0 = C_0 = 1$.

class (F), as it has been suggested that the potential for plasmids to hitchhike by being transmitted to such mutants plays an important role in their persistence (BERGSTROM *et al.* 2000). We developed a novel model on the basis of a combination of our previous analysis and the suggestions of BERGSTROM *et al.* (2000). Again, this demonstrates numerically plasmid persistence through damped oscillations, but at different equilibria from those observed without the adaptive mutant class (Figure 7).

DISCUSSION

Here we examine and expand the model of BERGSTROM *et al.* (2000) to clarify the conditions under which plasmids are predicted to be maintained in bacterial populations. Our initial, and most significant, finding was that the assumption of Stewart and Levin's criterion, which states that parasitic plasmids cannot persist within homogenous bacterial populations, does not hold in any case we have considered, and we can prove that it does not hold in the case of constant coefficients. Indeed a striking, and somewhat counterintuitive, inference from our reevaluation of BERGSTROM *et al.*'s (2000) equations is that plasmid-bearing cells can be maintained by oscillations with the plasmid-free class, even when the plasmid confers a significant fitness cost. The transmission rates used in our model are likely to be conservative, as recent reports have highlighted the role

of biofilms in maintaining high cell densities, and thus high transfer rates, in natural communities.

We went on to examine the dynamics of our model under conditions where plasmids confer a selective advantage to the cell and also considered the possibility that adaptive plasmid genes may be transferred to the chromosome. We note that plasmid gene transfer to the chromosome leads to plasmid-free cells that have not acquired the selective trait being driven to extinction. However, plasmid-bearing cells and plasmid-free cells that have acquired the adaptive gene will coexist by the same dynamic that plasmid-free and plasmid-bearing cells coexist in the original model (essentially, the effect of the adaptive plasmid gene is canceled out by transfer to the chromosome).

BERGSTROM *et al.* (2000) proposed a model where resource limitation was considered implicitly through coefficient functions varying in time. We modeled it explicitly by including carrying capacity as a parameter and showed that coexistence between plasmid-free and plasmid-bearing cells could still occur, via damped oscillations to a steady state rather than persistent undamped oscillations (stronger competition would lead to overdamping). In this model, plasmid-bearing cells are more likely to persist at high plasmid transfer rates. Recent reports have highlighted the role of biofilms in maintaining high cell densities and hence high transfer rates in natural communities. We might therefore expect to find examples when plasmid-bearing cells persist in biofilms but fail to persist elsewhere.

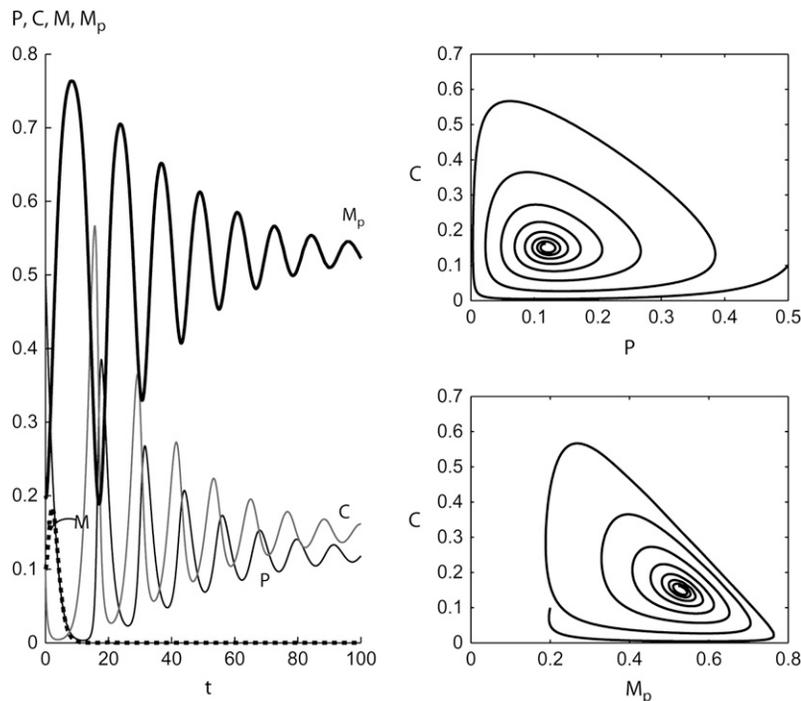


FIGURE 7.—Damped oscillations of plasmid persistence considering the introduction of an adaptive mutation to the plasmid-free class F .

Finally, again following the lead of BERGSTROM *et al.* (2000), we examined the effect of hitch-hiking. Once more, we note that this is not a prerequisite for plasmid persistence, but will simply change the dynamics such that plasmids will be maintained at a new equilibrium value. In sum, we find no evidence to support the claims that a strong selective advantage or population heterogeneity is required for the maintenance of plasmids in bacterial populations and argue instead that even costly plasmids may persist in homogenous populations through undamped or (more realistically) damped oscillations with the plasmid-free class.

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APPENDIX

The nontrivial steady state of the system (1),

$$\begin{aligned}\frac{dF}{dt} &= \beta F - \gamma PF - uF \\ \frac{dP}{dt} &= \beta(1-a)P + \gamma PF - \tau P - uP\end{aligned}$$

is $(F^*, P^*) = ((\tau + u - \beta(1-a))/\gamma, (\beta - u)/\gamma)$ and when calculated on the Jacobian of the system gives two imaginary eigenvalues, $\lambda_{1,2} = \pm i\sqrt{(\beta - u)[\tau + u - \beta(1-a)]}$.

Such an equilibrium is a neutrally stable steady state.

The nontrivial steady state of system (1) including the selective advantage of the plasmid genes $(1 + b)$,

$$\begin{aligned}\frac{dF}{dt} &= \beta F - \gamma PF - uF \\ \frac{dP}{dt} &= \beta(1-a)(1+b)P + \gamma PF - \tau P - uP\end{aligned}\tag{A1}$$

is now simply $(F^*, P^*) = ((\tau + u - \beta(1-a)(1+b))/\gamma, (\beta - u)/\gamma)$. Similarly to the previous situation, this equilibrium has an oscillatory behavior and leads to coexistence of both plasmid-free and plasmid-bearing cells.

The chromosomal class (C) , denoting bacterial cells where the adaptive plasmid genes have been integrated

into the chromosome, is incorporated into the previous system as follows:

$$\begin{aligned}\frac{dF}{dt} &= \beta F - \gamma PF - uF \\ \frac{dP}{dt} &= \beta(1-a)(1+b)P + \gamma P(F+C) - \tau P - uP \\ \frac{dC}{dt} &= \beta(1+b)C - \gamma PC - uC.\end{aligned}\tag{A2}$$

From the above, it can be shown that

$$\frac{1}{C(t)} \frac{dC(t)}{dt} - \frac{1}{F(t)} \frac{dF(t)}{dt} = \beta b \Rightarrow \frac{C(t)}{F(t)} = \frac{C(0)}{F(0)} e^{\beta b t}.$$

This demonstrates that we can omit the first equation, as the F class becomes negligible over time.

So, the nontrivial equilibrium of the resulting system (A3),

$$\begin{aligned}\frac{dP}{dt} &= \beta(1-a)(1+b)P + \gamma P(F+C) - \tau P - uP \\ \frac{dC}{dt} &= \beta(1+b)C - \gamma PC - uC\end{aligned}\tag{A3}$$

is $(P_1^*, C_1^*) = ((\beta(1+b) - u)/\gamma, (\tau + u - \beta(1-a)(1+b))/\gamma)$ and remains neutrally stable.