The ISME Journal (2009) 3, 761–769

www.nature.com/ismej

ORIGINAL ARTICLE

Competition favours reduced cost of plasmids to host bacteria

Rembrandt JF Haft, John E Mittler and Beth Traxler
Department of Microbiology, University of Washington, Seattle, WA, USA

Conjugative plasmids of Gram-negative bacteria have both vertical and horizontal modes of transmission: they are segregated to daughter cells during division, and transferred between hosts by plasmid-encoded conjugative machinery. Despite maintaining horizontal mobility, many plasmids carry fertility inhibition (*fin*) systems that repress their own conjugative transfer. To assess the ecological basis of self-transfer repression, we compared the invasion of bacterial populations by *fin*$^+$ and *fin*$^-$ variants of the plasmid R1 using a computational model and co-culture competitions. We observed that the *fin*$^+$ variant had a modest cost to the host (measured by reduction in growth rate), while the *fin*$^-$ variant incurred a larger cost. In simulations and empirical competitions the *fin*$^-$ plasmid invaded cultures quickly, but was subsequently displaced by the *fin*$^+$ plasmid. This indicated a competitive advantage to reducing horizontal transmission and allowing increased host replication. Computational simulations predicted that the advantage associated with reduced cost to the host would be maintained over a wide range of environmental conditions and plasmid costs. We infer that vertical transmission in concert with competitive exclusion favour decreased horizontal mobility of plasmids. Similar dynamics may exert evolutionary pressure on parasites, such as temperate bacteriophages and vertically transmitted animal viruses, to limit their rates of horizontal transfer.
The ISME Journal (2009) **3,** 761–769; doi:10.1038/ismej.2009.22; published online 2 April 2009
Subject Category: microbial population and community ecology
Keywords: conjugation; microbial ecology; parasite evolution; plasmid biology

Introduction

Gram-negative bacteria host a wide variety of plasmids that encode conjugative self-transfer systems (Lawley *et al.*, 2004). The ecology of these plasmids is of medical interest as they are of prime importance in the prevalence of drug resistance in bacterial populations (Levin and Andreasen, 1999; Mazel and Davies, 1999; Wise, 2004). Bacteria and their conjugative plasmids can also provide a tractable model of a host–parasite system. These genetic elements can persist in suitable hosts for many generations without conferring a selective advantage (Turner *et al.*, 2002; Dahlberg and Chao, 2003; De Gelder *et al.*, 2004; Bahl *et al.*, 2007).

Conjugative plasmids in Gram-negative bacteria are vertically transmitted to daughter cells during division, but also horizontally transmitted to neighbouring cells by self-encoded type IV secretion systems known as '*tra*' (for transfer) systems. The persistence and diversity of the *tra* systems suggests that their horizontal transfer capabilities have been valuable to plasmids over evolutionary time. However, many plasmids repress their own conjugative transfer with *cis*-encoded 'fertility inhibition' (*fin*) systems. *Fin* genes inhibit expression and assembly of their cognate *tra* systems, and have been described in Gram-negative plasmids spanning the F, I and X incompatibility (Inc) groups; IncP plasmids also carry genes that limit *tra* expression (Firth *et al.*, 1996; Lawley *et al.*, 2004). The effects of *fin* systems can be dramatic: the *finOP* system of the well-studied plasmid R1 reduces conjugative efficiency about 1000-fold (Dionisio *et al.*, 2002).

In essence, *fin* systems antagonize a plasmid's capacity for horizontal transfer; the conservation of *fin* genes suggests that they provide conjugative plasmids with an evolutionary advantage. Why would it be advantageous to repress horizontal transfer? Lundquist and Levin (1986) postulated that *fin* systems represent a compromise between the benefits of horizontal transfer (to the plasmid) and the associated costs to the host, which could manifest themselves in a variety of ways. Expression of *tra* genes makes cells susceptible to certain bacteriophages and can induce both cytoplasmic and extracytoplasmic stress–response pathways with pleiotropic effects on the host (Anderson, 1968; Campbell, 1996; Zahrl *et al.*, 2006). Avoiding

Correspondence: B Traxler, Department of Microbiology, University of Washington, Box 357242, Seattle, WA 98195-7242, USA.
E-mail: btraxler@u.washington.edu
Received 19 November 2008; revised 13 February 2009; accepted 17 February 2009; published online 2 April 2009

either of these factors might provide the evolutionary pressure for *tra* repression. Furthermore, increased horizontal transfer might reduce vertical transmission by slowing host growth; such tradeoffs have been predicted and observed for diverse parasites (Bull *et al.*, 1991; Kover *et al.*, 1997; Kover and Clay, 1998; Messenger *et al.*, 1999; Dahlberg and Chao, 2003; Jensen *et al.*, 2006). Plasmids display a tradeoff between conjugation rate and vertical transfer (Turner *et al.*, 1998; Turner, 2004), and have been shown to evolve reduced cost to the host at the expense of horizontal transfer when the environment limits conjugative spread (Dahlberg and Chao, 2003).

To assess the ecological role of transfer–repression systems, we computationally simulated competitions between *fin*$^+$ and *fin*$^-$ plasmids. We modelled an environment in which the plasmid offers no growth advantage to its host; the plasmid is, in this case, a parasitic genetic element. These simulations indicated a broad competitive advantage for *fin*$^+$ plasmids in the absence of phages or inducers of stress–response pathways, suggesting that the tradeoff between horizontal and vertical transmission is itself strong enough to select for the maintenance of *fin* systems. In addition to simulated competitions, we competed the naturally occurring *fin*$^+$ plasmid R1 with its *fin*-deficient derivative R1-16 in laboratory-grown populations of *Escherichia coli*. R1 and R1-16 compete through mutual exclusion from host cells, as the presence of one conjugative plasmid prevents entry by related plasmids (Clark, 1985; Firth *et al.*, 1996). Empirical competitions between R1 and R1-16 agreed well with our theoretical predictions and showed a competitive advantage for R1. Our results indicate that competition between plasmids for limited hosts exerts a downward evolutionary pressure on horizontal transfer rates, which helps explain the persistence of *fin* systems in nature. We hypothesize that vertical transfer and mutual exclusion play key roles in shaping the dynamics of plasmid competitions in nature.

Materials and methods

Growth media, bacteria and plasmids

Rich broth (LB) was used as a growth medium (Miller, 1972). Medium supplements were used at the following concentrations: 30 μg ml^{-1}, chloramphenicol (Cml); 40 μg ml^{-1}, kanamycin (Kan). *E. coli* were the K12-derived strain XK1502 (Panicker and Minkley, 1985). Plasmids were wild-type R1 (IncFII, transfer-proficient, *fin*$^+$ Kan^R Cml^R) and the R1-derived R1-16 (IncFII, transfer-proficient, *fin*$^-$ Kan^R Cml^S) (Blohm and Goebel, 1978). R1-16 carries the R1 surface exclusion system (Bayer *et al.*, 1995); we verified that R1 cannot efficiently conjugate into and be maintained in cells carrying R1-16 (data not shown).

Simulation design and programming

Our model was inspired by the mass-action chemostat models of Levin *et al.* (1979) and Hansen and Hubbell (1980), but adapted to serial-transfer experiments such as those performed by Turner (2004) and Dahlberg and Chao (2003). Our model considered the growth of bacterial populations in the presence of a limiting nutrient C, with periodic dilution into fresh medium with a nutrient concentration C_0 every 24 h. Simulated cultures contained up to four distinct populations: plasmid-free cells (N), cells hosting a *tra*-repressed plasmid P_{fin+} (P_{1b}), new recipients of P_{fin+} not yet repressed by *fin* (P_{1a}), and cells hosting a *tra*-derepressed plasmid P_{fin-} (P_2). Related plasmids prevent one another from entering the same cell by surface exclusion mechanisms (Clark, 1985; Firth *et al.*, 1996), so we assumed that cells carrying one plasmid would not be recipients in further conjugative transfers. Variables used in the model are defined in Table 1, and the differential equations that describe cell and resource concentrations are shown below.

Plasmid-free cells (N):

$$\begin{aligned} dN/dt =& (r_N N + r_{1a}s_1P_{1a} + r_{1b}s_1P_{1b} + r_2s_2P_2) \\ & \times (C/(C + K_m)) \\ & - (\gamma_{1a}P_{1a} + \gamma_{1b}P_{1b} + \gamma_2P_2)N \\ =& (\text{growth} + \text{segregation}) - (\text{conjugation}) \end{aligned}$$

Recent recipients of plasmid 1, not yet fin-repressed (P_{1a}):

$$\begin{aligned} dP_{1a}/dt =& (1 - s_1)(r_{1a}P_{1a})(C/(C + K_m)) \\ & + (\gamma_{1a}P_{1a}N) + (\gamma_{1b}P_{1b}N) - (f_1P_{1a}) \\ =& (\text{growth} - \text{segregation}) \\ & + (\text{conjugation}) - (\text{fin repression}) \end{aligned}$$

Cells carrying fin-repressed plasmid 1 (P_{1b}):

$$\begin{aligned} dP_{1b}/dt =& (1 - s_1)(r_{1b}P_{1b})(C/(C + K_m)) + (f_1P_{1a}) \\ =& (\text{growth} - \text{segregation}) + (\text{fin repression}) \end{aligned}$$

Cells carrying the fin-deficient plasmid 2 (P_2):

$$\begin{aligned} dP_2/dt =& (1 - s_2)(r_2P_2)(C/(C + K_m)) + (\gamma_2P_2N) \\ =& (\text{growth} - \text{segregation}) + (\text{conjugation}) \end{aligned}$$

Growth-limiting carbon source (C) (replenished every 24 h):

$$\begin{aligned} dC/dt =& - ((yC)/(C + K_m)) \\ & \times (r_N N + r_{1a}P_{1a} + r_{1b}P_{1b} + r_2P_2) \\ =& - (\text{consumption}) \end{aligned}$$

Transfer of diverse conjugative plasmids is inhibited by stationary phase and high-cell densities (Sudarshana and Knudsen, 1995; Frost and Manchak, 1998). To incorporate this dynamic into our simulations, we included a switch that reduced conjugation rates 1000-fold when C fell below 1/10th of the

Table 1 Definition of modelled parameters and their default values in simulations

Symbol	*Definition*	*Unit*	*Default*	*Source of default*
N	Density of plasmid-free cells	CFU ml^{-1}	—	—
P_{1a}	Density of cells bearing transitorily-derepressed P_{fin+}	CFU ml^{-1}	—	—
P_{1b}	Density of cells bearing P_{fin+}	CFU ml^{-1}	—	—
P_2	Density of cells bearing P_{fin-}	CFU ml^{-1}	—	—
C	Resource concentration	μg ml^{-1}	—	—
C_0	Starting resource concentration	μg ml^{-1}	200	—
r_N	Growth rate for N	h^{-1}	1.459	This study
r_{1a}	Growth rate for P_{1a}	h^{-1}	1.230	Inferred from r_2
r_{1b}	Growth rate for P_{1b}	h^{-1}	1.405	This study
r_2	Growth rate for P_2	h^{-1}	1.230	This study
γ_{1a}	Conjugation rate for P_{1a} donors	ml cell^{-1} h^{-1}	3.8×10^{-9}	Inferred from γ_2
γ_{1b}	Conjugation rate for P_{1b} donors	ml cell^{-1} h^{-1}	4.4×10^{-12}	Levin *et al.* (1979)
γ_2	Conjugation rate for P_2 donors	ml cell^{-1} h^{-1}	3.8×10^{-9}	Levin *et al.* (1979)
s_1	Segregation rate for P_{fin+}	—	1×10^{-4}	Olsson *et al.* (2004)
s_2	Segregation rate for P_{fin-}	—	1×10^{-4}	Olsson *et al.* (2004)
f_1	*fin* repression rate for P_{fin+}	—	0.1	Willetts (1974)
K_m	Monod constant	μg ml^{-1}	0.2	Kovářová *et al.* (1996)
y	Yield coefficient	μg CFU^{-1}	8.0×10^{-8}	This study[a]

[a]The yield coefficient (Y) was adjusted to match final bacterial densities to those observed empirically in culture.

K_m value (see Table 1 for variable definitions). To account for observed cell death in stationary phase (see Results), a 1-log drop in CFU ml^{-1} at the 24-h time point was incorporated into the computational simulations. Bloodshed Dev-C++ version 4.9.9.2 was used for programming and compiling computational simulations.

Determination of plasmid cost and stationary-phase survival

Plasmid cost was measured as the increase in generation time relative to the plasmid-free progenitor strain. Growth rates were measured by monitoring the OD_{600} of cultures growing in LB (without antibiotics) after 100-fold dilution from overnight cultures in LB (supplemented with Kan for strains bearing R1 or R1-16). Only time points corresponding to logarithmic growth were used in calculating doubling times. To monitor stationary-phase survival, overnight cultures of XK1502, XK1502 R1 and XK1502 R1-16 were diluted 1:10 in fresh LB. Viable cell counts were determined by plating aliquots of each culture at 0, 6, 12, 18 and 24 h post-dilution.

Plasmid invasion and competition experiments

Overnight cultures of XK1502 were diluted 1:100 into fresh LB and mixed with 1:10 000 dilutions of XK1502 R1 and/or XK1502 R1-16 overnight cultures. Cultures grew at 37 °C, in 10 ml glass culture tubes containing 2 ml volumes of culture, aerated by slow rolling (45 r.p.m.), and diluted 100-fold into fresh broth every 24 h. After 8 h, aliquots were removed to determine viable cell counts before stationary phase (see below for viable count method). Viable counts were also determined at 24, 48, 72 and 96 h.

For each time point, dilutions of the cultures were plated in quadruplicate to non-selective, Kan-containing and Cml-containing media. Cells containing R1 grew on all plates, cells containing R1-16 were killed by Cml, and plasmid-free cells were killed by both antibiotics. Colony counts from the three types of plates were used to calculate cell densities by the following formulae: R1-containing cells = Cml plate counts; R1-16-containing cells = Kan plate counts minus Cml plate counts; plasmid-free cells = non-selective plate counts minus Kan plate counts. This method, while repeatable, led to some uncertainty in plasmid-free cell numbers when they fell to 2% or less of plasmid-bearing levels. In ambiguous cases, colonies from non-selective plates were patched to Kan and Cml to more precisely determine the densities of plasmid-bearing and plasmid-free cells. On a few occasions a minority population was beneath the level of detection; we omitted these data points from the analysis (as noted in Figure 3 and its legend).

Results

Computational simulations predict a competitive advantage for fin*-mediated repression*

We examined the fitness of parasitic *fin*$^+$ and *fin*$^-$ plasmids by comparing their abilities to invade host populations, individually and in competitive co-cultures. We use the term 'invasion' to refer to the increase in prevalence of an initially rare plasmid in a host population, and the subsequent maintenance of that plasmid over time. Invasion as we define it can thus be achieved by horizontal transfer and/or vertical transmission of plasmids.

We first approached plasmid invasion using computational simulations. Our model considered two variants of a parasitic conjugative plasmid:

a fin^+ (transfer-repressed) plasmid P_{fin+} and a fin^- (transfer-derepressed) plasmid P_{fin-}. We used simulations to predict the ability of conjugative plasmids to invade a plasmid-free population in three distinct scenarios: P_{fin+} alone, P_{fin-} alone or both plasmids in co-culture. Initially, we set parameters to simulate fin^+ and fin^- variants of the naturally occurring IncFII plasmid R1 in a population of *E. coli*. R1 and its derivatives have been used in several studies on plasmid invasion (Levin *et al.*, 1979; Lundquist and Levin, 1986; Dionisio *et al.*, 2002; Dahlberg and Chao, 2003; Turner, 2004). Conjugation rates for such plasmids have been measured earlier (Levin *et al.*, 1979), and we empirically determined costs for R1 and the fin^- mutant R1-16 in an *E. coli* K12 strain (see below).

We simulated growth under relatively nutrient-rich conditions (resulting in stationary-phase cell densities similar to those observed in LB), with plasmid-bearing cells initially making up 1% of the population. The resultant simulations are shown as Figure 1. When present alone P_{fin+} and P_{fin-} both invaded a plasmid-free population, with P_{fin+} achieving a majority by day 3 and P_{fin-} achieving a majority in <1 day (Figures 1a and b). Plasmids were maintained over $\sim$3500 simulated generations, with plasmid-free cells persisting at a stable plasmid-specific level. When both plasmids were present together, P_{fin-} quickly assumed the majority, but within a few days P_{fin-} was overtaken by P_{fin+}; P_{fin+} went on to dominate the mixed culture in the long term (Figure 1c).

These results predict a long-term competitive advantage for P_{fin+} in spite of earlier takeover by P_{fin-}. However, these simulations represent only one of a variety of possible plasmid–host-environment combinations. The success of actual plasmid invasions is mainly determined by plasmid cost, host density and conjugation rates (Levin *et al.*, 1979; Lundquist and Levin, 1986; Turner, 2004; Bahl *et al.*, 2007). We therefore wished to determine and compare the effects of bacterial density and plasmid cost on invasion by P_{fin+} and P_{fin-}, which represent plasmids with differing conjugation rates.

To explore this wider parameter space, we first ran iterative simulations in which we varied the initial resource concentration C_0. Increasing C_0 increases the population density reached by simulated bacteria before they exhaust their limiting resource and stop dividing. We considered each plasmid separately and then both in co-culture. At the end of each simulation, we tallied one of three outcomes: no plasmid persisted, plasmid P_{fin+} persisted as the most numerous or plasmid P_{fin-} persisted as the most numerous. The results of these iterative simulations are summarized in Figure 2. Neither plasmid persisted at the lowest C_0 value, and increasing nutrient concentrations were advantageous for both plasmids (Figure 2a). Although P_{fin-} was the more costly plasmid, its high conjugation rate allowed it to invade cultures at $C_0 \geqslant 40$, whereas P_{fin+} could only invade at $C_0 \geqslant 100$. Populations containing both plasmids displayed a striking result: in environments where only P_{fin-} would have invaded alone, P_{fin+} dominated mixed cultures. In these competitions, P_{fin-} first spread through the population and P_{fin+} cells then displaced their less-fit P_{fin-} competitors, whereas uninfected cells were in the minority (data not shown). At intermediate resource levels ($40 \leqslant C_0 \leqslant 80$), this dynamic allowed P_{fin+} to invade mixed cultures when it could not have persisted alone. Resource-variant simulations thus indicated that, in contrast to single-plasmid systems, co-culture conditions broadly favoured vertical transfer over horizontal transfer.

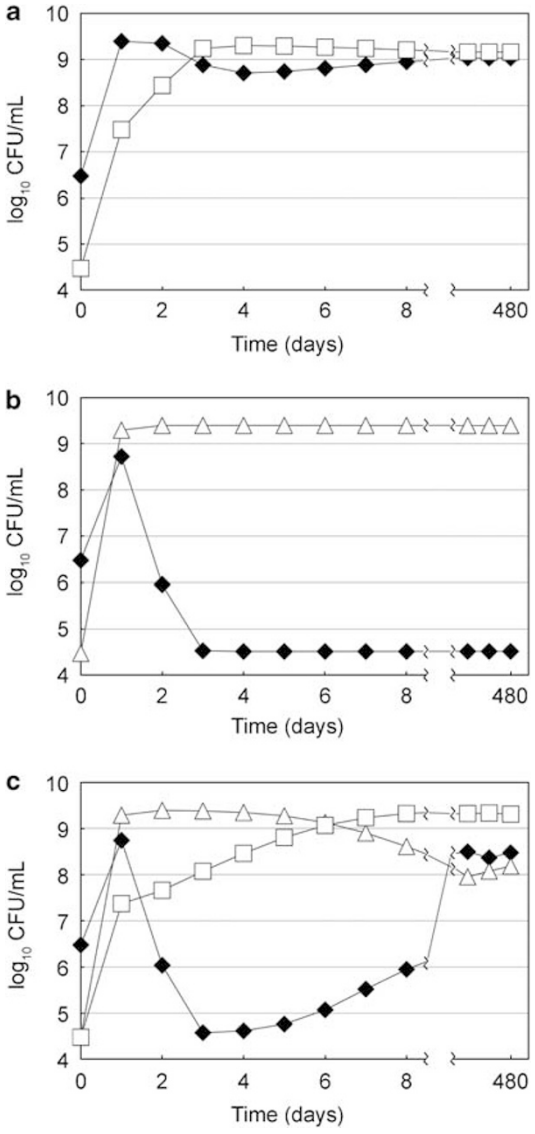

Figure 1 Predicted population dynamics in co-cultures with plasmid-free and plasmid-containing cells. Simulations were run for 500 days ($\sim$3500 generations). ◆, plasmid-free cells; □, P_{fin+}-containing cells; Δ, P_{fin-}-containing cells. (**a**) Co-cultures with P_{fin+}. (**b**) Co-cultures with P_{fin-}. (**c**) Co-cultures with P_{fin+} and P_{fin-}.

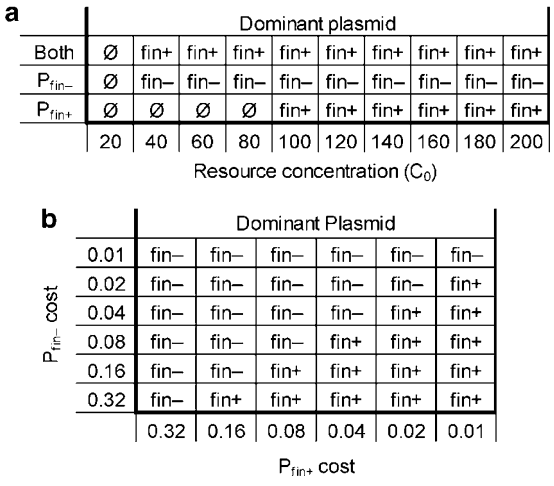

Figure 2 Simulations of plasmid invasion and competition. Each cell in a grid represents a single simulation run for 500 days (~3500 generations). '∅' = neither plasmid persisted; '*fin*+' = persistence of P_{fin+} as the majority plasmid; '*fin*–' = persistence of P_{fin-} as the majority plasmid. (**a**) Invasions and competitions at C_0 values from 20 to 200 μg ml^{-1} (representing maximal cell densities of 2×10^8 to 2×10^9 CFU ml^{-1}). (**b**) Competitions between P_{fin+} and P_{fin-} at $C_0 = 200$ μg ml^{-1} with varying plasmid costs (cost = 1−W, where W is the growth rate relative to a plasmid-free strain).

To investigate the role played by plasmid cost, we simulated cultures containing both P_{fin+} and P_{fin-} in the presence of a plasmid-free majority, varying the costs of each plasmid independently (and tallying the most numerous plasmid at the end of each simulation). We defined cost as the fractional reduction in growth rate of a plasmid-bearing strain relative to the plasmid-free strain. We set $C_0 = 200$, such that stationary-phase cell densities were comparable with those empirically observed in LB (see below), and both plasmids could invade given growth rate costs of <50%. When plasmid costs were equal, P_{fin-} spread rapidly through the population and was not displaced by P_{fin+} (Figure 2b). However, a very modest reduction in cost allowed P_{fin+} to outcompete P_{fin-}, in spite of the fact that P_{fin-} had a ~1000-fold greater conjugation rate. Even a cost reduction of 0.01 (the smallest interval tested) provided a clear advantage for P_{fin+}. These results supported our earlier observations that decreasing cost to the host provides a competitive advantage to a parasitic plasmid.

On the basis of our simulations, we were able to make a number of predictions about the behaviour of conjugative plasmids in bacterial populations, assuming horizontal transfer is costly to the host. A *fin*$^-$ plasmid should invade more quickly, and under a wider range of conditions, than a *fin*$^+$ relative. A *fin*$^+$ plasmid, however, should have a long-term competitive advantage over a *fin*$^-$ relative. And finally, the presence of a *fin*$^-$ plasmid may facilitate invasion by its *fin*$^+$ relative under certain conditions.

The fin$^+$ *plasmid R1 has a competitive advantage over its* fin$^-$ *mutant R1-16*

We tested our predictions using the conjugative plasmids, and we considered in our initial simulations: R1 and its *fin*-deficient derivative R1-16. R1 and R1-16 replicate stably in *E. coli*, and can be differentiated by selectable markers (R1 is Kan^R Cml^R, whereas R1-16 is Kan^R Cml^S). To aid in building our simulations, and in preparation for empirical tests, we determined the growth cost of each plasmid in *E. coli* growing in LB in the absence of antibiotics. In the K12 strain XK1502, doubling times during logarithmic growth were as follows: XK1502, 28.5 ± 0.2 min; XK1502 R1, 29.6 ± 0.3 min; XK1502 R1-16, 32.0 ± 0.7 min (data are the mean ± s.e. of three biological replicates). Increased doubling times of plasmid-containing strains indicate that both R1 and R1-16 are costly to the host, with R1-16 being the more costly plasmid.

Our simulations using these growth costs predicted that R1 and R1-16 would individually invade plasmid-free populations, and in a mixed culture R1-16 would invade cultures rapidly only to be overtaken by R1 (Figure 1). To test these predictions, we performed plasmid invasion/competition assays with R1 and R1-16. We did not select for plasmid maintenance; under these conditions, plasmid costs should be equal to those we measured empirically. Three different experimental scenarios were tested, corresponding to the three scenarios simulated computationally (see Figures 1 and 2): R1 only; R1-16 only; or both plasmids together.

The results of these competition experiments are shown in Figure 3. Our experimental data agreed well with the simulated competitions. For each plasmid individually, horizontal transfer was sufficient to overcome plasmid cost in dense *E. coli* cultures. Alone, R1 invaded and was present in the majority of cells after 3 days, whereas R1-16 alone achieved majority within a day; these dynamics agree almost exactly with our predictions (compare Figures 1 and 3). Our prediction that in a mixed culture R1-16 would invade rapidly and then be outcompeted by R1 was also borne out in these experiments. Population dynamics in experimental cultures were highly repeatable, with all four biological replicates of each competition following the same path as the mean presented here (data not shown).

One aspect of the actual competition between R1 and R1-16 that differed from our computational predictions was the rapidity of R1 takeover after R1-16 invasion (in comparison with simulations with P_{fin+}/P_{fin-}; compare Figure 1c with Figure 3c). One clue to this disparity is the behaviour of the competitors between the 8 and 24 h time points: XK1502 R1-16 densities decreased significantly more than XK1502 R1 densities between 8 and 24 h in the co-culture competitions (Figure 3c; R1 slope = 0.007, R1-16 slope = −0.034, d.f. = 3, $P = 0.04$). In these co-culture competitions,

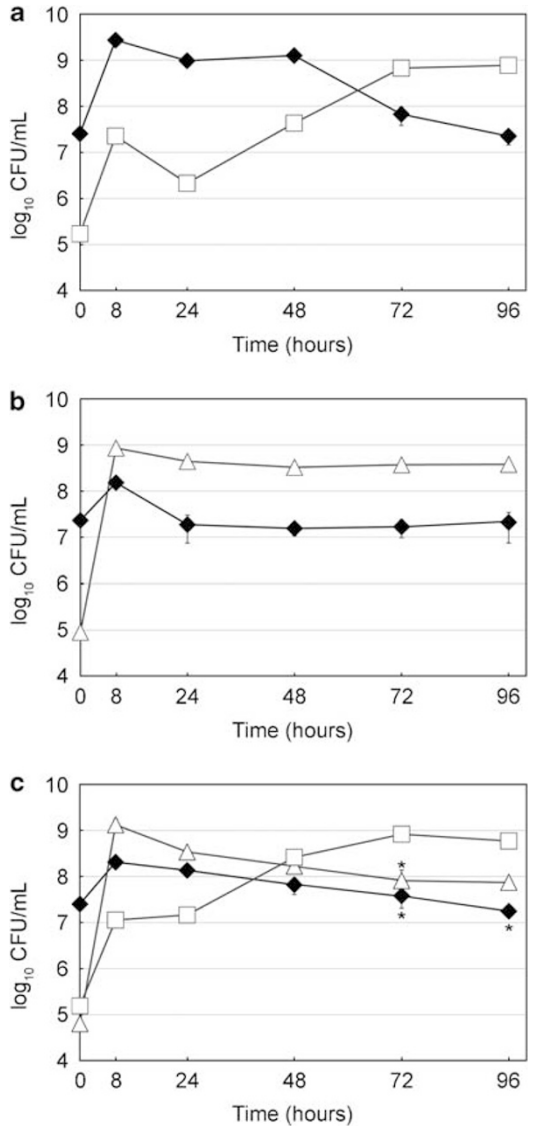

Figure 3 Population dynamics in co-cultures with plasmid-free and plasmid-containing cells. All experiments began by mixing a minority of plasmid-bearing cells with a 100-fold excess of plasmid-free cells. ◆, plasmid-free cells; □, R1-containing cells; △, R1-16-containing cells. Vertical bars denote s.e. of four biological replicates; bars smaller than the associated data point are omitted. (**a**) Co-cultures with R1. (**b**) Co-cultures with R1-16. (**c**) Co-cultures with R1 and R1-16. Asterisks highlight points for which one or two replicates were beneath the level of detection. These points represent the mean of detectable isolates only and may be an overestimate of the true mean.

R1-bearing cells survived the 8–24 h period better than in R1-only invasion experiments (Figure 3a; R1 slope $= -0.030$, Figure 3c; R1 slope 0.007, d.f. $= 6$, $P = 0.004$). Computational simulations indicated that this increase in survival could account for the relatively rapid timing of R1 takeover in the presence of R1-16 (data not shown).

Why R1-bearing cells survived stationary phase so well in these competitions is unclear. In control experiments, monitoring stationary-phase survival, R1-bearing cells survived no better than plasmid-free or R1-16-bearing cells (data not shown). R1-bearing cells died normally in the competitions in which R1-16 was not present (Figure 3a), ruling out the possibility that our R1-bearing strain had acquired a GASP phenotype (Zambrano and Kolter, 1996; Zahrl *et al.*, 2006) or some similar mutation that gave it a general advantage under stationary-phase conditions. Because IncF conjugation is strongly inhibited during stationary phase (Frost and Manchak, 1998), the disparity in plasmid persistence probably did not arise from plasmid transfer events. We posit instead that R1-bearing cells had an advantage in survival or proliferation over R1-16-bearing cells. This could have resulted from environmental modifications by cells carrying R1-16 that disproportionately benefited cells carrying R1. One candidate effect of R1-16 that we noted in our assays was the formation of macroscopic cellular aggregates when cells carrying R1-16 reached high densities in liquid media (data not shown). While stationary-phase clumping would not *a priori* benefit R1-bearing cells, it may have given them some unexpected advantage in competitions.

In summary, both R1 and R1-16 invaded plasmid-free populations even though they were demonstrably costly under the growth conditions used. The loss of *fin*-mediated *tra* repression allowed R1-16 to invade a plasmid-free population quickly, but put it at a competitive disadvantage in the presence of R1, its wild-type parent with a functioning *fin* system.

Discussion

Our results shed light on a conundrum in plasmid evolution: why plasmids do not maximize their own conjugative transfer rates. Several groups have seen plasmid-bearing populations evolve to minimize plasmid cost to the host, which is generally associated with a reduction in conjugation rates (Freter *et al.*, 1983; Turner *et al.*, 1998; Dahlberg and Chao, 2003; Turner, 2004; Dionisio *et al.*, 2005). Furthermore, many natural plasmids repress expression of their self-transfer functions (Firth *et al.*, 1996; Lawley *et al.*, 2004). These observations are surprising given that increased rates of conjugation aid plasmids in invading and persisting within host populations, as demonstrated by this work and others' (Stewart and Levin, 1977; Dionisio *et al.*, 2002; De Gelder *et al.*, 2007, 2008; Miki *et al.*, 2007). Here we show that, in a population of plasmid-bearing cells, a variant plasmid with a decreased conjugation rate can give its hosts a competitive edge. Increased replication of hosts carrying low-conjugation plasmid variants can thus explain the experimental observations of conjugation rate reduction and the evolutionary persistence of *fin* systems in conjugative plasmids of Gram-negative bacteria. Cells bearing fin^+ plasmids grow more quickly than cells carrying fin^- relatives, outcompeting them and increasing the prevalence of the fin^+ plasmid. The extreme of this dynamic is represented

in some of our simulated competitions, which suggest that under certain culture conditions P_{fin+} would be unable to invade unless P_{fin-} were present to help it become established (Figure 2a, $40 \leqslant C_0 \leqslant 80$).

One notable result we did not predict was that R1-bearing cells enjoyed increased survival during stationary phase if (and only if) R1-16-bearing cells were present at a high density (see Results). To the best of our knowledge, this interaction between hosts carrying competing R plasmids has not been described earlier. Unexpected interactions such as this highlight the importance of empirical competitions, even when good theoretical predictions are available. The reasons behind the preferential survival of R1-bearing cells are unclear, and can be addressed in future work.

It is important to note that various aspects of plasmid ecology not considered by our model might influence the outcome of plasmid competitions in natural communities. Our experimental system allows bacteria to spread and propagate in new environments, but does not include immigration of new strains into established populations. Although we observe a benefit associated with *fin* systems, *fin*-deficient plasmids might be favoured if there were a regular influx of plasmid-free recipients (Turner *et al.*, 1998; Bergstrom *et al.*, 2000). This sort of dynamic may explain the existence of naturally *tra*-derepressed conjugative plasmids such as F, which carries an IS3 insertion in its *finO* gene (Firth *et al.*, 1996). However, we believe that natural environments would generally increase the advantageousness of *fin* genes for the following reasons. (i) Derepressed *tra* expression in *E. coli* cells has been shown to elicit stress responses, indicating pleiotropic negative effects of *fin* mutants on their hosts (Zahrl *et al.*, 2006). (ii) Many plasmids carry genes such as antibiotic-resistance markers that make them advantageous under certain conditions (Eberhard, 1990); sporadic selection for plasmids would help them rise to high levels in a population, which we have shown favours *tra* repression. Transient selection can also allow plasmids to evolve increased stability in non-optimal hosts (De Gelder *et al.*, 2008). (iii) The presence of plasmid-specific phages in natural environments would increase the fitness of *tra*-repressed cells by preferentially killing highly piliated *fin* mutants (Anderson, 1968; Campbell, 1996). Furthermore, other environmental variables not present in our model may allow fin^+ plasmids to attain high rates of transfer, potentially increasing their invasion potential in the absence of fin^- competitors. Variations in host strains allow some donors to be functionally *fin*-deficient (Dionisio *et al.*, 2002; Dionisio, 2005; Harr and Schlotterer, 2006). Also, structured environments allowing the development of aggregates or biofilms can increase the spread of wild-type conjugative plasmids in bacterial populations (Molin and Tolker-Nielsen, 2003). Our model assumes mass-action kinetics associated with well-mixed populations; the kinetics of plasmid spread can be quite different on surfaces (Simonsen, 1990; Krone *et al.*, 2007; Fox *et al.*, 2008).

Returning from the specific to the general, our results are consistent with the proposal that *fin* systems represent a compromise between the interests of the plasmid and those of the host (Lundquist and Levin, 1986). Characteristics of the plasmid–host relationship that set the stage for this compromise are the obligate intracellular nature of plasmids, the pairing of vertical and horizontal modes of transfer and the surface exclusion. Future experiments could determine which of these is most important in biasing the system towards a competitive reduction in plasmid transfer. Such work would provide insights that might help to understand and control the spread of antibiotic resistance in clinically relevant environments.

This work also has implications outside of plasmid biology, in which our mathematical model could apply to any host–parasite system that fits its basic assumptions. In general terms, we predict that a parasite may be selected for in the environment if it provides protection from a more virulent pathogen. The competitive advantage we observed for a low-cost (that is, low-virulence) parasitic element provides a valuable contrast to studies that predict or demonstrate an increase in virulence under competitive pressure for other parasites (Kover *et al.*, 1997; Kover and Clay, 1998; Kerr *et al.*, 2006; Coombs *et al.*, 2007; Bull and Ebert, 2008). We postulate that the importance of vertical transmission and the resistance of the infected host to superinfection are key factors that support a competitive reduction in virulence in our system. Theoretical work suggests that frequent vertical inheritance under competitive conditions might limit virulence in other parasites as well (Frank, 1996). Understanding factors that drive evolution of virulence or temperance will provide insights into the spread of infectious diseases and allow us to better determine which infectious agents require particular attention as new pathogens emerge.

Acknowledgements

Ellen Zechner kindly provided plasmids R1 and R1-16. We thank Gene Nester, Eva Top and Stephen Krone for critical reading of the manuscript. This work was supported by the National Science Foundation (MCB-0345018). R Haft was supported in part by the National Institute of General Medical Sciences (NRSA T32 GM07270).

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