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Source-sink plasmid transfer dynamics maintain gene mobility in soil bacterial communities

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Horizontal gene transfer is a fundamental process in bacterial evolution that can accelerate adaptation via the sharing of genes between lineages. Conjugative plasmids are the principal genetic elements mediating the horizontal transfer of genes, both within and between bacterial species.In some species, plasmids are unstable and likely to be lost through purifying selection, but when alternative hosts are available, interspecific plasmid transfer could counteract this and maintain access to plasmid-borne genes. To investigate the evolutionary importance of alternative hosts to long-term plasmid population dynamics in an ecologically relevant environment we established simple soil microcosm communities comprising two species of common soil bacteria, *Pseudomonas fluorescens* and *Pseudomonas putida*, and a mercury resistance (Hg^R) plasmid, pQBR57, both with and without positive selection (i.e. addition of Hg(II)). In single-species populations, plasmid stability varied between species: while pQBR57 survived both with and without positive selection in *P. fluorescens*, it was lost or replaced by non-transferrable Hg^R captured to the chromosome in *P. putida*. A simple mathematical model suggests these differences were likely due to pQBR57's lower intraspecific conjugation rate in *P. putida*. By contrast, in two-species communities, both models and experiments show that intraspecific conjugation from *P. fluorescens* allowed pQBR57 to persist in *P. putida* via source-sink transfer dynamics. Moreover, the replacement of pQBR57 by non-transferrable chromosomal Hg^R in *P. putida* was slowed in co-culture. Interspecific transfer allows plasmid survival in host species unable to sustain the plasmid in monoculture, promoting community-wide access to the plasmid-borne accessory gene pool and thus potentiating future evolvability.

Horizontal gene transfer | plasmids | mobile genetic elements | microbial ecology

INTRODUCTION

Horizontal gene transfer (HGT) is a key process in bacterial evolution, driving the spread of ecologically and clinically important traits such as resistances to environmental toxins and antibiotics (1). Conjugative plasmids are extrachromosomal genetic elements that carry genes for their horizontal transfer between bacteria (i.e. conjugation) and are principal mediators of HGT both within and between species (2, 3). Because plasmid-borne 'accessory genes' (i.e. genes not directly involved in core plasmid functions) can enhance the virulence, metabolism or resistance of bacterial hosts (1), the population dynamics of plasmids is fundamentally important to understanding bacterial adaptation (3).

Plasmids impose costs on their hosts (4), and theory suggests that without positive selection for accessory genes, plasmids should be lost from bacterial populations due to purifying selection unless counteracted by a high rate of conjugation (5, 6). Under positive selection, plasmids should also eventually be lost as selection favours chromosomal integration of accessory genes and loss of the redundant plasmid (5). In addition to the immediate loss of accessory genes, the loss of conjugative plasmids from populations decreases the potential for HGT, thereby diminishing a key mode for acquisition of novel adaptive genes and thus limiting bacterial evolvability.

Several mechanisms could act to maintain plasmids. Compensatory evolution can ameliorate plasmid cost, thereby weakening selection against the plasmid (7-9). However, this process is unlikely to stabilise highly unstable plasmids or maintain plasmids in small populations where the rate of plasmid loss is likely to exceed the rate of compensatory evolution. Plasmids may carry genes that directly enhance their stability, such as partitioning genes or toxin-antitoxin systems, but even when present such systems are imperfect, resulting in plasmid-free segregants (10). Plasmids can also be maintained within a host species as infectious elements, provided conjugation rates are high (e.g. (11)).

An alternative mechanism is for plasmid loss in a focal host species to be counteracted by on-going transfer from another species in which the plasmid is stably maintained. Such interspecific conjugation, analogous to transmission of infectious disease from a reservoir host (12), could maintain access to the mobile gene pool, allowing the focal species to remain evolutionarily responsive to temporally or spatially variable selection (3). Plasmids can be shared by a considerable fraction of the microbial community (13), but surprisingly there have been few experimental tests of how the presence of alternative hosts affects plasmid population dynamics, particularly over periods longer than a few days. Moreover, most studies of plasmid dynamics have been performed in well-mixed rich laboratory media, which do not adequately represent the physical structure or nutrient availability in most natural microbial communities (14, 15). Structured communities may present fewer opportunities for plasmid donors to encounter recipients, but clustering of genotypes in

Significance

Bacterial adaptation through horizontal gene transfer is central to microbial evolution, and in the context of antibiotic resistance represents a growing clinical threat. Conjugative plasmids are key mediators of genetic exchange both within and between species. Experimental studies have mostly focused on plasmid population dynamics in single-species populations, but between-species transfer could counteract purifying selection and maintain plasmids in hosts that would otherwise lose them. We show that plasmids can be lost from single-species populations, even when their genes are under selection, because beneficial genes are captured by the chromosome. In contrast, experiments and models show that in a two-species community, between-species transfer maintains community-wide access to plasmids, promoting the spread of the ecologically and clinically important genes they carry.

Reserved for Publication Footnotes

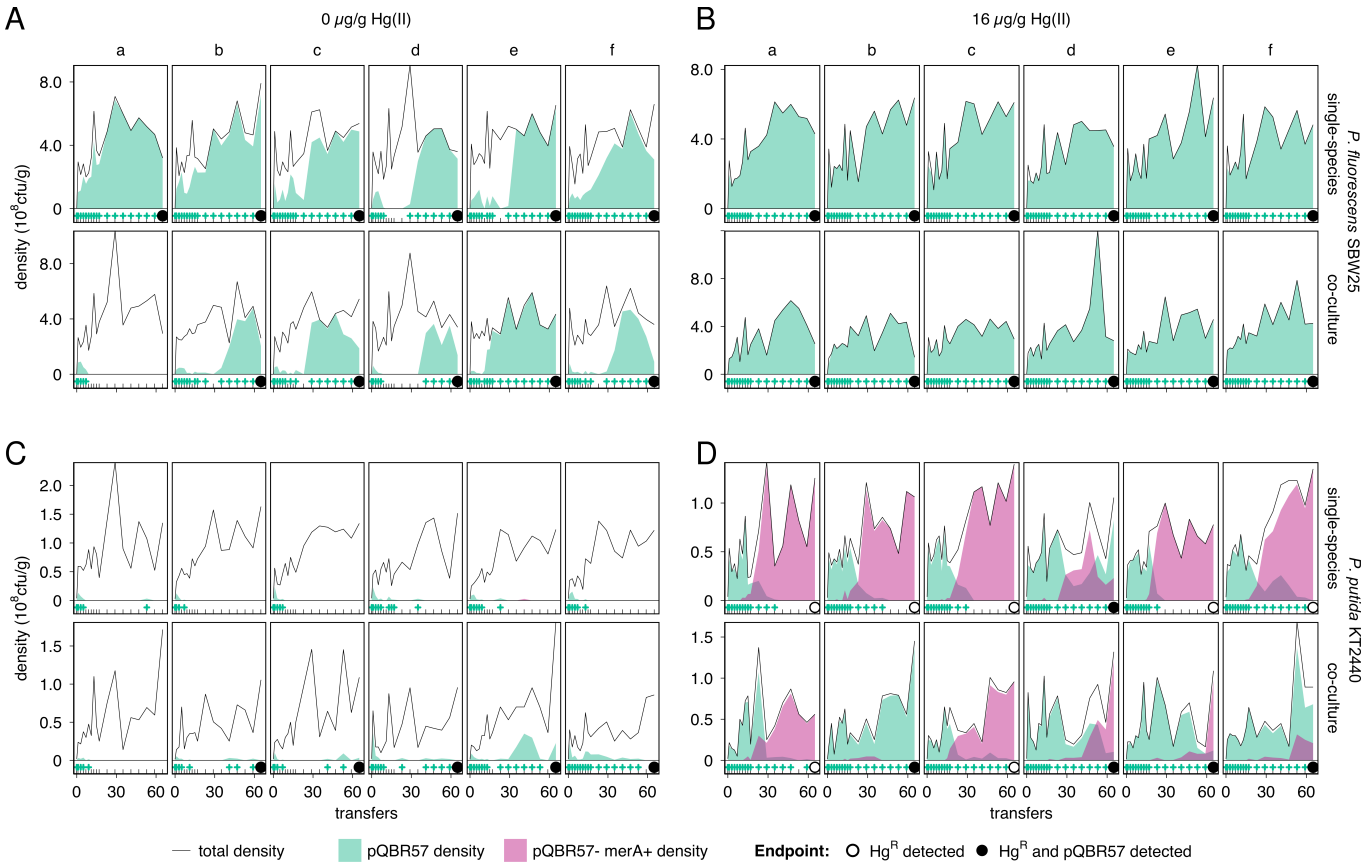

Fig. 1. Co-culture with favourable host *P. fluorescens* promotes plasmid carriage in unfavourable *P. putida* (A) *P. fluorescens* populations evolved with 0 μg/g Hg(II). The upper row of sub-panels shows single-species populations; the lower row shows populations cultured alongside *P. putida* (co-culture). Six replicate populations (columns, labelled a–f) were initiated for each treatment. Each sub-panel shows, for an individual population, total density at transfer (solid line), the density of pQBR57+ (filled green area below the line), and the density of pQBR57– *merA*+ mutants (filled purple area below the line). For clarity, tick marks at the bottom of each sub-panel indicate sampling times and green '+' symbols indicate detection of pQBR57. A black circle at the final sampling point (transfer 65) indicates that Hg^R remained in the population at the end of the experiment; filled circles indicate pQBR57 (and Hg^R) remained. Note that no pQBR57– *merA*+ mutants were detected in *P. fluorescens*. (B) *P. fluorescens* populations evolved with 16 μg/g Hg(II). As panel A, except evolved with 16 μg/g Hg(II). (C) *P. putida* populations evolved with 0 μg/g Hg(II). As panel A except populations were *P. putida*. The lower row of sub-panels shows populations cultured alongside *P. fluorescens* (co-culture). Each population of co-cultured *P. putida* a–f was grown with the corresponding co-cultured *P. fluorescens* population (a–f, panel A). (D) *P. putida* populations evolved with 16 μg/g Hg(II). As panel C, except evolved with 16 μg/g Hg(II). Different y-axis scales are used for each species: *P. fluorescens* density was ∼5x *P. putida*.

space may promote species coexistence (16) and also allow plasmids to rapidly sweep through naïve recipient populations once encountered (17, 18).

To test how the presence of alternative host species affects plasmid population dynamics we established populations of *Pseudomonas fluorescens* SBW25 and *Pseudomonas putida* KT2440 either individually ('single-species'), or together ('co-culture'), in sterile soil microcosms, which offer a spatially structured, low resource and near-natural environment (19). Pseudomonads such as *P. fluorescens* and *P. putida* are widespread and often coexist in natural soil communities (20). Populations were founded with a mercury resistance (Hg^R) plasmid (the 307 kb pQBR57, isolated from the same site as *P. fluorescens* SBW25 (21)) at ∼50% starting-frequency, with approximately equal numbers of pQBR57-bearers (pQBR57+) in each species for the co-culture treatment. Every four days, samples were transferred into fresh microcosms which had either been pre-treated with selective levels of mercuric chloride (16 μg/g Hg(II)) or with an equal volume of water (0 μg/g Hg(II)). Such transfers represent a simple controllable regime which acts as a proxy for the dynamic 'turnover' of nutrients occurring in soil habitats (22), and 16 μg/g Hg(II) corresponds to specific mercury contamination, such as in industrial or post-industrial sites (23). The dynamics of the bacterial populations, the frequency of pQBR57, and the frequency of the mercury reductase gene (*merA*) were tracked over 65 transfers (approximately ∼440 generations, SI Text).

RESULTS

Plasmid dynamics were strongly affected by host species and culture conditions

The dynamics of pQBR57 varied greatly between species and with Hg(II) treatment. pQBR57 was generally maintained in *P. fluorescens* under both Hg(II) treatments, going extinct in only one replicate (replicate a, 0 μg/g Hg(II), co-culture). Under 0 μg/g Hg(II) (Figure 1A), plasmid frequencies were variable between replicates and across time, particularly during the early part of the experiment. No significant effect of living alongside *P. putida* could be detected in terms of pQBR57 survival (Fisher's Exact Test, $p = 1$), constancy (Wilcoxon Signed-Rank Test, $Z = 0$, $p = 1$) or dynamics (GLMM, effect of co-culture, parametric bootstrapping $p = 0.08$). Under 16 μg/g Hg(II), both in one-species and co-culture treatments (Figure 1B), pQBR57 fixed in *P. fluorescens* by transfer five and remained so until the end of the experiment. *P. fluorescens* was therefore a favourable host for pQBR57, in that it generally maintained the plasmid regardless of selective environment.

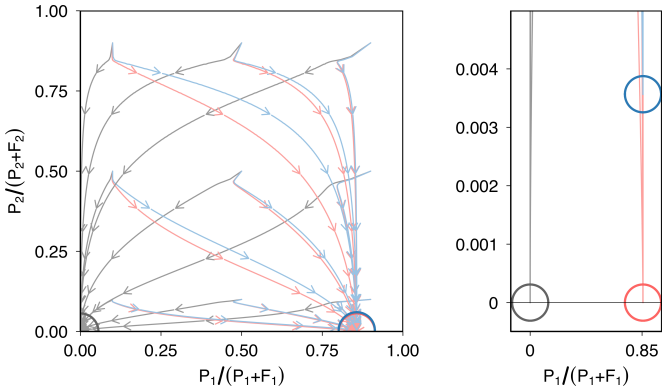

Fig. 2. A two-species model predicts between-species conjugation can promote plasmid carriage in an unfavourable host species. (A) Plasmid frequency in species 1 (*P. fluorescens*-like, , x-axis) and species 2 (*P. putida*-like, , y-axis) was simulated over 5000 iterations of a simple mass-action plasmid dynamics model. The model was initiated with varying plasmid starting frequencies (0.1, 0.5 and 0.9). Arrows indicate the passage of time for each simulation, and a coloured circle indicates the final state. Models omitting conjugation (grey) result in the loss of plasmid from both species. Models omitting interspecific conjugation (red) result in plasmid maintenance in species 1, but extinction in species 2, whereas models including interspecific conjugation (blue) result in plasmid maintenance at low levels in species 2. (B) Zoomed view of panel A. With interspecific conjugation, the plasmid is maintained at approximately 0.35% in species 2.

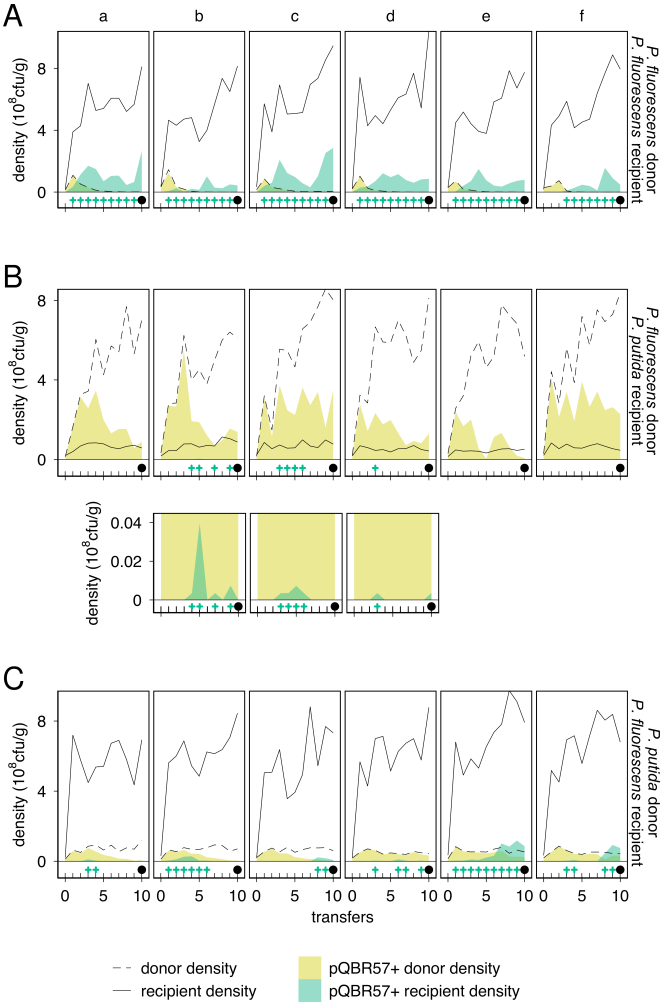

Fig. 3. Short term experiments show maintenance of pQBR57 by conjugation. (A) *P. fluorescens* donor and *P. fluorescens* recipient. Six replicate populations (columns, a–f) were initiated for each treatment. Each sub-panel shows the densities at transfer of bacteria that began with pQBR57 ('donors', dashed line) and bacteria that began without pQBR57 ('recipients', solid line). The density of pQBR57+ is shown for the donors (filled yellow area below the dashed line) and the recipients (filled green area below the solid line). At the bottom of each sub-panel, ticks indicate sampling points, green '+' symbols indicate detection of plasmid-bearing recipients, and a black circle indicates detection of plasmid-bearing recipients at the end of the experiment. (B) As panel A, except the donor species was *P. fluorescens* and the recipient species was *P. putida*. The smaller sub-panels below replicates b, c and d show zoomed regions of the upper sub-panels to indicate low frequency pQBR57+ *P. putida*. (C) As panel A except with *P. putida* donor and *P. fluorescens* recipient.

In contrast, pQBR57 was poorly maintained in single-species *P. putida* populations. In the 0 µg/g Hg(II) single-species treatment (Figure 1C, upper row), pQBR57 decreased rapidly in frequency and ultimately went extinct in all replicates, resulting in a completely Hg(II)-sensitive population. In the 16 µg/g Hg(II) single-species treatment (Figure 1D, upper row), pQBR57 frequency increased to near-fixation in all populations before transfer five. However, mutants that lost pQBR57 but retained the mercury reductase *merA* gene (pQBR57– *merA*+) soon emerged and reached high frequency (>50%) in all populations. In 5/6 replicates pQBR57– *merA*+ mutants eventually outcompeted plasmid bearers, resulting in plasmid extinction by the end of the experiment. In single-species populations, therefore, pQBR57 was significantly more likely to go extinct when its host was *P. putida* rather than *P. fluorescens*, both under parasitic 0 µg/g Hg(II) (Fisher's Exact Test, p = 0.0022) and mutualistic 16 µg/g Hg(II) (p = 0.015) conditions. *P. putida* was therefore an unfavourable pQBR57 host, in that it generally lost the plasmid regardless of selective environment.

However, living in co-culture with *P. fluorescens* had a positive effect on pQBR57 carriage by *P. putida* under both Hg(II) conditions. In 0 µg/g Hg(II) (Figure 1C, lower row), 5/6 co-cultured *P. putida* populations carried pQBR57 at detectable levels during the experiment, particularly in two replicates (e and f). Control experiments, in which we mixed plasmid-containing *P. fluorescens* and plasmid-free *P. putida* immediately before spreading on selective media, did not yield any transconjugants (SI Text), suggesting that these clones carried pQBR57 *in situ* rather than acquiring it on the surface of the agar plate. pQBR57 therefore benefitted from a reduced chance of extinction in co-cultured *P. putida* in 0 µg/g Hg(II) (Fisher's Exact Test, p = 0.015), and we detected a positive effect of co-culture on the frequency of *P. putida* plasmid-carriers over time (GLMM, effect of co-culture:transfer, parametric bootstrapping p = 0.025; effect of co-culture p = 0.006). The exception was replicate a, in which pQBR57 also went extinct in the co-cultured *P. fluorescens* population.

In 16 µg/g Hg(II) (Figure 1D, lower row), like with single-species culture, pQBR57– *merA*+ mutants arose in all co-cultured *P. putida* populations. However in 2/6 co-cultured populations these mutants remained ≤ 30%, and in one replicate (b) they were subsequently lost. Overall, the presence of *P. fluorescens* had a positive effect on the frequency of plasmid-carriage in *P. putida* in 16 µg/g Hg(II) (GLMM, effect of co-culture:transfer, parametric bootstrapping p = 0.045; effect of co-culture p = 0.008), though we did not detect a significant difference in plasmid extinction between single-species and co-cultured *P. putida* (Fisher's Exact Test, p = 0.24), probably because strong selection for Hg^R, and hence pQBR57 initially, resulted in high frequencies of pQBR57+ *P. putida* in all populations in the early part of the experiment. Co-culturing with the favourable host *P. fluorescens* therefore enhanced plasmid presence in the unfavourable host *P. putida*, both when plasmid-borne genes were

under positive selection (16 μg/g Hg(II)) and when the plasmid was parasitic (0 μg/g Hg(II)).

pQBR57 was sustained by conjugative transfer

Within species, theory predicts that variation in plasmid dynamics is determined by the net cost of carriage and the rates of conjugative transfer and segregational loss (5, 6). Differences in pQBR57 stability between *P. putida* and *P. fluorescens* cannot be explained by costs, because we found pQBR57 to be more costly in *P. fluorescens,* which maintained the plasmid, than in *P. putida*, which did not (SI Text). In contrast, we found that pQBR57 had a relatively high intraspecific conjugation rate in *P. fluorescens*, approximately 1000x that in *P. putida* (SI Text), which might explain maintenance and spread of pQBR57 in *P. fluorescens* without positive selection. Furthermore, we could detect interspecific transfer of pQBR57 in both directions (SI Text). If pQBR57 could be maintained in *P. fluorescens* by intraspecific conjugation, then in co-culture *P. fluorescens* might act as a source for *P. putida* through interspecific conjugation. Alternatively, variation in the rate at which plasmid-free segregants arise (segregation rate) may explain differences in plasmid maintenance between the species.

To explore the role of these processes, we first tested the effect of conjugation in a simple mass-action model of plasmid dynamics (24) adapted to include two species. For species 1, the change in the number of plasmid-free bacteria F_1 over time is given by

$$\frac{dF_1}{dt} = (\alpha_1 F_1 + \delta P_1)\left(1 - \frac{(F_1+P_1)}{K_1}\right) - \gamma_{11}F_1P_1 - \gamma_{21}F_1P_2 - \mu F_1 \quad (1)$$

and the change in the number of plasmid-containing-bacteria P_1 is given by

$$\frac{dP_1}{dt} = (\beta_1 P_1 - \delta P_1)\left(1 - \frac{(F_1+P_1)}{K_1}\right) + \gamma_{11}F_1P_1 + \gamma_{21}F_1P_2 - \mu P_1 \quad , (2)$$

where α_1 is the species 1-specific plasmid-free growth rate, β_1 is the species 1-specific plasmid-bearing growth rate, γ_{11} is the species 1 intraspecific conjugation rate, γ_{21} is the interspecific conjugation rate from species 2 to species 1, K_1 is the species 1-specific carrying capacity, δ is the plasmid segregation rate and μ is the washout rate. Similar equations were written using the species 2-specific parameters to describe the dynamics of F_2 and P_2, with intraspecific conjugation rate γ_{22} and interspecific conjugation rate from species 1 to species 2, γ_{12}. Because we did not detect a significant effect of co-culture on the growth rates or carrying capacities of *P. fluorescens* or *P. putida* (SI Text) we assumed that interspecific competition did not greatly affect growth dynamics. Parameter estimates were obtained experimentally for *P. fluorescens* ('species 1') and *P. putida* ('species 2', see SI Text) where possible, and the four-equation model run with varying starting plasmid frequencies for 5000 iterations either with interspecific and intraspecific conjugation, with intraspecific conjugation only, or without any conjugation. To test the robustness of the qualitative model predictions we also ran the model with sets of parameters randomly drawn from a wide range of plausible values (Supplementary Figures S1–S3). The model with no conjugation ultimately saw plasmid extinction in both species (Figure 2). With intraspecific conjugation the plasmid stabilised at ~85% in species 1, although it went extinct in species 2. Importantly, adding interspecific conjugation allowed plasmid persistence in both species, albeit at low frequency in species 2 (~0.35%, Figure 2B). Further exploration of the parameter space showed that plasmid survival in species 1 was due to higher levels of intraspecific conjugation, which in turn was due to conjugation rate and to a lesser extent the larger population size of species 1 (Figure S1), while plasmid survival in species 2 depended on plasmid survival in species 1 and interspecific conjugation from species 1 to species 2 (Figure S2). Segregation rates, however, could be varied over a large range without qualitative effect on the model predictions, suggesting the observed plasmid dynamics are better explained by intra- and interspecific conjugation (Figure S3).

The mass-action model is a simple approximation of the ecological system and hence excluded many details; most notably the spatial structure inherent to soil. Therefore, to explicitly test the predicted importance of conjugation in plasmid maintenance we ran short-term experiments using marked strains to follow the densities and plasmid status of bacteria beginning with ('donors') and without pQBR57 ('recipients'). In single-species *P. fluorescens* populations (*P. fluorescens* donor and recipient, Figure 3A), consistent with the cost of pQBR57, we found that donors were rapidly outcompeted by recipients, and were not detected in any replicate by the end of the experiment (10 transfers). However, the plasmid was maintained in all replicates at ~20-30% due to transfer into the recipient strain. These results, qualitatively consistent with the mass-action model, show that pQBR57 survival in *P. fluorescens*, at least in the short term, was through conjugative transfer. To test whether co-habiting with plasmid-bearing *P. fluorescens* promoted plasmid carriage in *P. putida* we co-cultured recipient *P. putida* with donor *P. fluorescens*. Consistent with the model results we found plasmid-bearing *P. putida* at low frequencies both during the experiment (3/6 replicates) and at the end of the experiment (6/6 replicates, Figure 3B). Interestingly, despite beginning the experiment at a plasmid frequency of 100%, plasmid carriage in *P. fluorescens* was reduced to ~25% by the end of the experiment, demonstrating the emergence of, and selection for, plasmid-free segregants. We also tested whether co-habiting with donor *P. putida* allowed pQBR57 invasion of a plasmid-free *P. fluorescens* recipient population. In all replicates we detected plasmid-bearing *P. fluorescens* (Figure 3C), and in two replicates, e and f, plasmid carriage by *P. fluorescens* reached frequencies sufficient for prolonged maintenance (as determined by comparison with Figure 3A). In contrast we saw marked plasmid loss from *P. putida* in all replicates due to competition from plasmid-free segregants. These data are therefore not consistent with an alternative hypothesis: that pQBR57 maintenance in *P. putida* in co-culture was due to some other interspecific interaction (e.g. plasmid-borne genes that provide a selective advantage to *P. putida* only alongside *P. fluorescens*). Although mass-action models are more commonly used to describe liquid cultures, our ability to capture the qualitative features seen in the soil microcosms is consistent with reports that spatial structure has little effect on plasmid transfer dynamics when donor and recipient bacteria encounter each other early in the growth cycle (17). Together these results show that conjugative transfer underlies the invasion and maintenance of mobile resistance genes in a favourable bacterial host, and in so doing allows neighbouring, unfavourable host species sustained access to those genes.

Interspecific plasmid transfer can maintain gene mobility in unfavourable host species

In multi-species communities, favourable hosts could act as 'sources' of plasmid for other community members. To explore the effects of a plasmid source on a neighbouring species we adapted our model for a single focal species by replacing the explicit interspecific conjugation term $\gamma_{21}P_2$ in equations (1) and (2) with a rate constant Γ, representing the sum of all interspecific conjugation events with an external (fixed) population. This gives equations (3) and (4), allowing analytic progress (SI Text)

$$\frac{dF}{dt} = (\alpha F + \delta P)\left(1 - \frac{(F+P)}{K}\right) - \gamma FP - \Gamma F - \mu F \quad (3)$$

$$\frac{dP}{dt} = (\beta P - \delta P)\left(1 - \frac{(F+P)}{K}\right) + \gamma FP + \Gamma F - \mu P \quad .(4)$$

Without a plasmid source ($\Gamma = 0$), plasmid frequency in the focal species is determined primarily by the balance of the plasmid cost and the (intraspecific) conjugation rate. Under most parameter

combinations the plasmid either fixes or is completely lost, and with only a very narrow region of parameter space that results in a mixed population of plasmid-bearing and plasmid-free individuals (Figure S4). Adding a plasmid source ($\Gamma > 0$) eliminates the region of parameter space in which the plasmid is absent from the focal species, and expands the region resulting in plasmid fixation in the focal species (Figure S4). A plasmid source increases the effective conjugation rate for the focal species; in the simplified case where segregation is neglected, this increase is linear with the interspecific conjugation rate Γ (SI Text)

Next, we considered when plasmid-borne genes are under positive selection but can be captured by the chromosome at a low rate ϕ to produce chromosomal mutants, which benefit from the captured genes regardless of whether they also carry the plasmid. We expanded equations (3) and (4) and added two further equations to describe plasmid-free and plasmid-bearing chromosomal mutants ($\mathcal{C}$ and $\mathcal{Q}$ respectively) (25)

$$\frac{dF}{dt} = (\alpha F + \delta P)\left(1 - \frac{(F+P+\mathcal{C}+\mathcal{Q})}{K}\right) - \gamma F(P+\mathcal{Q}) - \Gamma F - \eta F - \mu F \quad (5)$$

$$\frac{dP}{dt} = (\beta P - \delta P)\left(1 - \frac{(F+P+\mathcal{C}+\mathcal{Q})}{K}\right) + \gamma F(P+\mathcal{Q}) + \Gamma F - \phi P - \mu P \quad (6)$$

$$\frac{d\mathcal{C}}{dt} = (\alpha \mathcal{C} + \delta \mathcal{Q})\left(1 - \frac{(F+P+\mathcal{C}+\mathcal{Q})}{K}\right) - \gamma \mathcal{C}(P+\mathcal{Q}) - \Gamma \mathcal{C} - \mu \mathcal{C} \quad (7)$$

$$\frac{d\mathcal{Q}}{dt} = (\beta \mathcal{Q} - \delta \mathcal{Q})\left(1 - \frac{(F+P+\mathcal{C}+\mathcal{Q})}{K}\right) + \gamma \mathcal{C}(P+\mathcal{Q}) + \Gamma \mathcal{C} + \phi P - \mu \mathcal{Q} \quad (8)$$

where $-\eta F$ represents selection against plasmid-free bacteria that do not have the beneficial genes (24). Similar to the case without positive selection, without a plasmid source the plasmid either remains at fixation in the focal species or is lost by competition with plasmid-free chromosomal mutants, with a narrow range of parameter values resulting in a mixed population of plasmid-bearers and plasmid-free chromosomal mutants (Figure S4). The addition of a plasmid source expands the region of parameter space that results in a mixed population in the focal species by inhibiting fixation of plasmid-free chromosomal mutants (Figure S4). Therefore the presence of a plasmid source in a microbial community is expected to greatly enhance persistence of plasmid-borne genes and maintenance of interspecific gene mobility.

DISCUSSION

We have shown that co-culture with an alternative host promoted the survival of a conjugative plasmid, maintaining community-wide access to the plasmid-borne gene pool. In single-species cultures, the plasmid invaded and was maintained by infectious conjugative transfer in one host (*P. fluorescens*), but was lost by segregation and purifying selection from the other (*P. putida*), regardless of whether its accessory genes were under selection. Co-culture enabled a 'source-sink' relationship in which interspecific transfer from the 'source' host *P. fluorescens* maintained the plasmid in the 'sink' host *P. putida*, preserving access to the accessory genes the plasmid carries. Long term plasmid stability varies widely even between strains of the same species (26), but source-sink transfer dynamics mean that if a conjugative plasmid is maintained in one member of a community, that member can become a plasmid source persistently infecting neighbouring sink species. In natural communities, plasmid maintenance was found to correlate with existing plasmid prevalence, suggesting a tendency of certain hosts to preferentially act as plasmid sources (27). This dynamic, in which a subset of a multi-host community is critical for persistence of an infectious element, is well studied in the context of disease reservoirs (12), and adapting theoretical and methodological approaches from disease reservoir ecology to plasmid biology could be productive, for example in identifying putative source species and understanding their role in the dissemination of important bacterial traits, like antibiotic resistance.

Potential plasmid recipients can stretch across diverse microbial groups (13), and although transconjugants within sink-species may be transient (due to segregation or purifying selection) (28) their continual replenishment by conjugation from the source means that microbial community richness may be more robust to occasional bouts of selection for plasmid-borne genes. Co-culture enhanced plasmid persistence in the sink species even under Hg(II) selection, whereas in single-species *P. putida* cultures, plasmid-carriers tended to be outcompeted by mutants with chromosomal Hg^R. Plasmid survival under positive selection has important consequences because plasmids can carry many accessory genes (e.g. (29)) not all of which are selected at any given time. Interspecific conjugation also provides opportunity for plasmid recombination with resident genetic elements, enhancing genomic diversification (2). Furthermore, prolonged source-sink transfer dynamics could promote plasmid host range expansion (30), as also shown for bacteriophage (31). Previously, Dionisio and colleagues (32) noted how multi-species communities might accelerate plasmid spread when a highly conjugative intermediate species enhances plasmid transfer between two poorly-conjugative species. In species-rich host communities this 'amplification effect' likely acts in concert with the source-sink transfer dynamic, with plasmid sources acting both as a conduit for rapid plasmid spread and a reservoir for long-term maintenance.

Conjugation rate rather than fitness cost explained differences in plasmid stability between the two pseudomonads. The plasmid was more readily lost from *P. putida* despite lower cost-of-carriage, presumably because less intraspecific conjugation meant plasmid-free individuals were less likely to be (re-)infected. Since conjugation depends on population density as well as intrinsic conjugation rate (18) the higher density of *P. fluorescens* may also have enhanced plasmid spread. Increases in density over the course of the experiment, perhaps due to adaptation to the growth medium and/or transfer regime, may partly explain the re-invasion of pQBR57 in *P. fluorescens* in several populations between transfers 13 and 41. Mutations can increase conjugation rate (32, 33), and transient derepression of transfer gene expression following plasmid acquisition can also accelerate plasmid spread through naïve host populations (e.g. (34)), an effect particularly pronounced for bacteria growing on surfaces (17), although it is unclear whether either of these mechanisms are at work here. It is relevant that within-species conjugation underlies pQBR57 persistence in *P. fluorescens*, because the source-sink transfer dynamic would be unavailable to a plasmid that ameliorated its cost by completely abrogating conjugation (e.g. (35)). However, high conjugation rate is not essential for a plasmid source: hosts that achieve long-term plasmid stability through other routes, such as compensatory evolution (9, 36), could also become sources, provided they retain some degree of interspecific conjugation.

It is tempting to explain the persistence of plasmids and other mobile genetic elements by the benefits they bring to a bacterial community, for example as a communal gene pool (3) or by increasing robustness to environmental uncertainty (14). However it is hard to envisage how selection might maintain mobile elements for the benefit of the community in the long term if they are costly for the individual cell in the short term (5). Our data shows community-wide access to beneficial accessory genes resulting from processes occurring in one species in that community, specifically the persistence of a conjugative plasmid by infection. This extends previous evidence demonstrating the invasion and survival of plasmids as infectious parasitic elements, especially in spatially structured populations (11, 26, 37).

Detailed molecular and genetic studies of plasmid-host adaptation are revealing the mechanisms behind plasmid stability (7, 9, 35, 38, 39). However, these studies have primarily been conducted in one plasmid / one host systems, which are not reflective of natural microbial populations containing many different bacterial species (40) and mobile genetic elements (21, 41). We have shown that even simple two-species microbial communities offer evolutionary opportunities unavailable in a single-species population. In a diverse community, a few bacterial species acting as stable sources of conjugative plasmids may represent hubs of horizontal gene exchange. Identifying those species and understanding their ecology could have important implications for the control of clinically important mobile elements.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Pseudomonas fluorescens SBW25 and *P. putida* KT2440, labelled with gentamicin or streptomycin resistance markers and either plasmid-free or carrying pQBR57, were used for experiments (21). Soil microcosms were established and maintained similarly to previously described (21) and 1% w/v soil wash was transferred to a fresh microcosm every 4 days. Viable counts of each species were obtained by spreading samples on media containing species-selective antibiotics. For the first experiment, plasmid status in each species was assessed by PCR on ∼30 colonies using primers targeting plasmid loci and the *merA* gene (SI Text). For the short-term experiment we assessed plasmid status by replica plating onto Hg(II) plates and tested representative colonies by PCR. To test for Hg^R at the end of the experiments we also spread samples on Hg(II) plates containing species-selective antibiotics and tested representative colonies by PCR. For the 16 µg/g treatment we sampled up to 64 colonies. Because we tested approximately the same number of colonies from each species, differences in population size between the two species did not affect detection limits.

Analysis and statistics

For analysis of plasmid dynamics, we cropped data collected before transfer 7 because plasmid frequencies were dynamic due to short-term ecological processes (e.g. selection for Hg^R causing plasmid fixation in Hg(II) treatments). Plasmid constancy was calculated using the Fluctuation Index (42) and analysed by Asymptotic Wilcoxon Mann-Whitney Rank Sum Tests. To compare plasmid dynamics we used the R package 'lme4' (43) to fit Generalised Linear Mixed Effects Models (GLMM) with binomial response distributions and logit link functions (44, 45). For end-point analyses, we compared populations using Fisher's Exact Test. Full details and R code can be found in SI Text. Analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria) and plots were created using 'ggplot2' (46). For the mathematical models, parameters were estimated experimentally where possible (SI Text), numerical solutions were found using MATLAB (Mathworks, Natick MA, U.S.A.), and analytic investigations performed with the help of Mathematica (Wolfram, Champaign IL, U.S.A.).

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