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Article:
Hall, James Peter John, Wood, Andrew James orcid.org/0000-0002-6119-852X, Harrison, Eleanor et al. (1 more author) (2016) Source-sink plasmid transfer dynamics maintain gene mobility in soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America. pp. 8260-8265. ISSN 1091-6490

https://doi.org/10.1073/pnas.1600974113
Source-sink plasmid transfer dynamics maintain gene mobility in soil bacterial communities

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Submitted to Proceedings of the National Academy of Sciences of the United States of America

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\textsuperscript{2+}) 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\textsuperscript{2+} 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 interspecific 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\textsuperscript{2+} 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.

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 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 ongoing 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.
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 (Hg³⁺) 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 one replicate (replicate a, 0 µg/g Hg(II), 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 Hg³⁺ remained in the population at the end of the experiment; filled circles indicate pQBR57 (and Hg³⁺) 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*.

![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 Hg³⁺ remained in the population at the end of the experiment; filled circles indicate pQBR57 (and Hg³⁺) 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*.](image-url)
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. 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 of selective media, 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 mutosaic 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 benefited 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. putida population.

In 16 µg/g Hg(II) (Figure 1D, lower row), like with single-species treatments, 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-carrying 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 Hg²⁺, 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 (segregative 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 $N_1$ over time is given by

$$\frac{dN_1}{dt} = (\alpha_1 \beta_1 + \delta_1)(1 - \frac{N_1}{K_1}) - \gamma_1 N_1 P_1 - \delta_2 N_1 P_2 - \mu N_1$$

and the change in the number of plasmid-containing bacteria $N_2$ is given by

$$\frac{dN_2}{dt} = (\delta_2 + \beta_2)(1 - \frac{N_2}{K_2}) + \gamma_1 N_1 P_1 + \gamma_2 N_2 P_2 - \mu N_2$$

where $\alpha_1$ is the species 1-specific plasmid-free growth rate, $\beta_1$ is the species 1-specific plasmid-bearing growth rate, $\gamma_2$ is the species 1-specific carrying capacity, $\delta_1$ is the plasmid segregation rate and $\delta_2$ is the washout rate. Similar equations were written using the species 2-specific parameters to describe the dynamics of $N_2$ and $P_2$, with intraspecific conjugation rate $\gamma_2$ and interspecific conjugation rate from species 1 to species 2, $\gamma_1$. 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 $\gamma_2 P_2$ in equations (1) and (2) with a rate constant $r$, representing the sum of all interspecific conjugation events with an external (fixed) population. This gives equations (3) and (4), allowing analytic progress (SI Text)

$$\frac{dN_1}{dt} = (\alpha_1 \beta_1 + \delta_1)(1 - \frac{N_1}{K_1}) - \gamma_1 N_1 P_1 - \delta_2 N_1 P_2 - \mu N_1$$

$$\frac{dN_2}{dt} = (\delta_2 + \beta_2)(1 - \frac{N_2}{K_2}) + \gamma_1 N_1 P_1 + \gamma_2 N_2 P_2 - \mu N_2$$

Without a plasmid source ($r = 0$), 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
mixed population of plasmid-bearing and plasmid-free individ-
uals (Figure S4). Adding a plasmid source (T \geq 0) eliminates the
region of parameter space in which the plasmid is absent from the
central 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 (SI Text).

Next, we considered when plasmid-borne genes are under
positive selection but can be captured by the chromosome at
a low rate \textit{e} 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 ($\xi$ and $\zeta$ respectively) (25)

\[
\frac{d \zeta}{dt} = (\zeta + \beta \Phi) \left( \frac{1 - (\gamma P + \delta Q)}{\Phi} \right) - \gamma \zeta (P + Q) - \Gamma \zeta - \eta \zeta - \mu \zeta
\]  
(5)

\[
\frac{d \eta}{dt} = (\gamma P - \delta \Phi) \left( \frac{1 - (\gamma P + \delta Q)}{\Phi} \right) + \gamma \Phi (P + Q) + \Gamma \eta + \delta \eta + \mu \eta
\]  
(6)

\[
\frac{d \xi}{dt} = (\xi + \Phi \Phi) \left( \frac{1 - (\gamma P + \delta Q)}{\Phi} \right) - \gamma \xi \left( P + Q \right) - \Gamma \xi - \eta \xi - \mu \xi
\]  
(7)

\[
\frac{d \eta}{dt} = (\beta Q - \delta P) \left( \frac{1 - (\gamma P + \delta Q)}{\Phi} \right) - \gamma \Phi (P + Q) + \Gamma \eta + \delta \eta + \mu \eta
\]  
(8)

where $-\eta$ 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).

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 conjugal plasmid, maintaining community-
wide access to the plasmid-borne gene pool. In single-species
cultures, the plasmid invaded and was maintained by infectious
conjugal 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 inter-
specific 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 conjugal 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 dis-
semination 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 purification 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 $Hg^A$. Plasmid survival under positive selec-
tion 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 opportu-
nity 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,
Domingo and colleagues (32) noted how long-term communities
might accelerate plasmid spread when a highly conjuga-
tive 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 mainte-
nance.

Conjugation rate rather than fitness cost explained differ-
ences in plasmid stability between the two pseudomonads. The
plasmid was more readily lost from $P. putida$ despite lower cost-
of-carryage, 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 in-
trinsic 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 pQBRS7 in $P. fluorescens$ in several populations
between transfers 13 and 41. Mutations can increase conjugation
rate (32, 33), and transient derepression of transfer gene expres-
sion following plasmid acquisition can also accelerate plasmid
spread through naive host populations (e.g. (34)), an effect parti-
cularly 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 pQBRS7
persistence in $P. fluorescens$, because the source-sink transfer dy-
namic 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 conjugal 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).

Footline Author
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 or few strains / 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. Identifying those species and understanding their ecological relationships could have important implications for the control of clinically important mobile elements.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Plasmid status in each strain 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 for Hg(II) resistance. For the long-term experiment we also spread colonies 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 in population size between the two treatments we did not affect detection limits.

Analysis and statistics

To analyze plasmid dynamics, we cropped data collected before transfer 7 because plasmid frequencies were dynamic due to short-term ecological processes (e.g. selection for Hg6 causing plasmid fixation in Hg(II) treatments). Plasmid constancy was calculated using the Fluuctuation Index (42) and verified by Asymptotic Wilcoxon Mann-Whitney Rank Sum Tests. To compare plasmid dynamics we used the R package "ime4" (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 means 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 (Springer Science & Business Media, New York).

ACKNOWLEDGEMENTS.

We thank V. Friman and J. Pitchford for comments. This work was supported by ERC Consolidator Grant Agreement no. 311490-COEVOCON to MAB.

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