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Title: Positive selection inhibits gene mobilisation and transfer in soil bacterial communities

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Main text:

Horizontal gene transfer (HGT) between bacterial lineages is a fundamental evolutionary process that accelerates adaptation. Sequence analyses show conjugative plasmids are principal agents of HGT in natural communities. However, we lack understanding of how the ecology of bacterial communities and their environments affect the dynamics of plasmid-mediated gene mobilisation and transfer. Here we show, in simple experimental soil bacterial communities containing a conjugative mercury resistance plasmid, the repeated, independent mobilisation of transposon-borne genes from chromosome to plasmid, plasmid to chromosome, and, in the absence of mercury selection, interspecific

gene transfers from the chromosome of one species to the other via the plasmid. By reducing conjugation, positive selection for plasmid-encoded traits, like mercury resistance, can consequently inhibit HGT. Our results suggest that interspecific plasmid-mediated gene mobilisation is most likely to occur in environments where plasmids are infectious, parasitic elements rather than those where plasmids are positively selected, beneficial elements.

Conjugative plasmids — semi-autonomous mobile genetic elements that transfer between bacteria — are key agents of horizontal gene transfer (HGT) [1,2], facilitating rapid bacterial adaptation by spreading ecologically important traits between lineages [3]. The physical movement or duplication of genes (gene mobilisation) between chromosomes and plasmids, and their subsequent transfer between hosts, can be decisive in microbial evolution, and has facilitated acquisition of antimicrobial resistance [4,5] and emergence of virulent pathogens [6,7]. Such mobilisation can be facilitated by transposable elements (TEs), which encode enzymes (transposases) allowing transfer of genes between replicons [2]. However, the effects of ecological factors and natural selection on gene mobilisation and subsequent HGT is unclear. This is particularly the case for natural environments with a high degree of spatial structure [8], which is rarely captured by experimental studies. An outstanding question is how positive selection for plasmid-encoded traits, like resistance genes, affects the ability of that plasmid to spread genes through a community. Although positive selection can favour HGT by benefitting bacteria which have acquired the plasmid (transconjugants), it can also prevent HGT by killing or inhibiting growth of potential plasmid recipients [9,10].

Experimental evolution of bacterial communities is a powerful tool for exploring the evolutionary dynamics of plasmid-mediated HGT, bridging the gap between simplified short-term laboratory studies [11,12] and comparative genomics of natural populations [4,5,13,14]. To investigate how positive selection for plasmid-borne genes and the presence of an alternative host species interact to determine plasmid dynamics, we established communities of the common soil bacteria *Pseudomonas fluorescens* and *Pseudomonas putida*, either alone or in co-culture, in sterilized soil microcosms, which offer a complex, spatially-structured, and experimentally tractable environment [15-17]. Communities began with the 307-kb conjugative plasmid pQBR57 at ~50% frequency; this plasmid was isolated from agricultural soil and carries a 7-kb mercury resistance (Hg^R) TE, Tn5042. We also established control communities without pQBR57. Microcosms were supplemented with mercuric chloride to 16 μg/g Hg(II)), or an equivalent volume of water (0 μg/g Hg(II)). This level of mercury contamination, similar to that found in industrial or post-industrial sites [18], selects for specific Hg^R but does not necessitate it [16]. Six replicate populations for each combination of treatments were grown for ~440 generations. Analysis of plasmid frequency dynamics showed that pQBR57 was generally maintained by *P. fluorescens* and lost by *P. putida*, but persisted in *P. putida* when co-cultured with *P. fluorescens*, due to interspecific 'source-sink' plasmid transfer [17]. To investigate consequent effects on gene mobilisation and transfer we sequenced clones from the beginning and the end of the experiment, and used the Bacterial and Archaeal Genome Analyser (BAGA) pipeline to identify structural variations [19].

Results:

We detected multiple, independent gene mobilisation events between plasmid and chromosome in each species. Strikingly and unexpectedly, we also identified numerous interspecific transfers of chromosomal genes in the co-cultured treatments, facilitated by pQBR57 (Fig. 1). Gene transfer from *P. fluorescens* to *P. putida* was exemplified by a previously-unannotated *P. fluorescens* TE, Tn6291, in *P. putida* plasmids from 2/6 co-cultured communities (Fig. 1, replicates b and f). Subsequent PCR analyses found Tn6291 in *P. putida* clones from 2 further co-cultured communities (replicates d and e). Tn6291, a 22 kb TE carrying 25 predicted open reading frames and located between 2060105 and 2082440 in the *P. fluorescens* SBW25 reference sequence (ENA identifier AM181176, part of genomic island SBW_GI-1 (ref. 20)), carries an array of cargo genes with putative cytochrome c/d oxidase functions. The presence of Tn6291 in *P. putida* indicates these genes mobilised from the *P. fluorescens* chromosome onto pQBR57, and subsequently transferred between species via conjugation. All Tn6291+ P. putida clones were also positive for pQBR57, suggesting Tn6291 remains plasmid-borne in these isolates. Indeed, we detected plasmid-borne Tn6291 in 4/6 0 μg/g Hg(II) single-species *P. fluorescens* populations, and BLAST analysis shows a similar transposon in another soil Pseudomonad, *P. syringae* pv. *syringae* B301D (ref. 21) , suggesting Tn6291 mobilises readily.

We also detected gene transfer from *P. putida* to *P. fluorescens*. *P. fluorescens* clones from 3/6 co-cultured populations had acquired the well-described *P. putida* TE Tn4652 (ref. 22) in their chromosomes, with plasmid-borne Tn4652 also present. Tn4652 is a 17

kb TE closely related to the Tn4651 toluene degradation transposon and encodes various putative enzymes including a diacylglycerol kinase and a sulfatase. Tn4652 mobilisation to the plasmid occurred readily, with events already detectible in the ancestral *P. putida* clones used to inoculate the soil and begin the experiment (see Materials and Methods). PCR analysis of clones obtained over the course of the evolution experiment detected Tn4652+ plasmids in *P. fluorescens* as early as transfer 3, however Tn4652 insertion in the *P. fluorescens* chromosome was only detected later, after transfer 41 (Supplementary Table 2). In all cases, Tn4652 inserted in a region of the *P. fluorescens* chromosome with atypical sequence composition, likely to be recently acquired DNA [20].

Importantly, interspecific transfer of chromosomal TEs via the plasmid was only detected in populations grown without positive selection for the plasmid (0 μg/g Hg(II)). The amount of plasmid conjugation occurring, and thus opportunities for interspecific gene transfer, is likely a function of the densities of plasmid bearers and recipients [23,24]. By killing potential plasmid recipients, mercury selection reduces encounters between plasmid donors and recipients, and therefore conjugation [9]. Indeed, short-term experiments examining pQBR57 transfer (Figure 2) showed reduced effects of conjugation on plasmid dynamics when the plasmid was under selection [10], implying limited gene exchange. Together, these data suggest that positive selection for plasmid-borne resistance genes reduced the ability of that plasmid to facilitate HGT of chromosomal genes.

While mercury selection reduced TE transfer between species, we detected frequent mobilisation of the Hg^R TE Tn5042 from pQBR57 to the chromosome. Single-species *P. putida* populations tended to lose the plasmid [17], and sequences show that under mercury selection this was facilitated by acquisition of chromosomal Tn5042. To track the acquisition of chromosomal Tn5042 by *P. putida* populations, we designed PCR primers targeting 'focal' Tn5042 insertions (i.e. insertions detected in the end-point genome sequences) in eight different *P. putida* populations, and applied these to clones collected across the experiment. As with single-species *P. putida* populations, chromosomal Tn5042 was readily acquired by co-cultured *P. putida*, although these populations tended to also maintain pQBR57 (Figure 3). Similarly, chromosomal Tn5042 was detected in the *P. fluorescens* chromosome, which maintained the plasmid, despite its redundancy [17]. These findings suggest that long-term plasmid maintenance largely depends on community context and on compatibility between plasmid and host, and, provided there are no restrictions on recombination of plasmid genes into the chromosome, is unlikely to be secured by positive selection for accessory genes alone [17,25,26].

Tn5042 also mobilised in the mercury-free treatments: we detected three instances of Tn5042 multiplying on plasmids, and one instance of Tn5042 copying to the *P. fluorescens* chromosome (which occurred by transfer 35, see Figure 3). Tn5042 insertions sometimes occurred multiple times in a lineage — in co-cultured *P. putida* with 16 µg/g Hg(II), one clone (from replicate c) ultimately carried six copies. Although Tn5042 copy number increased in some clones from the mercury-free treatment, we detected more copies in clones evolved under mercury selection ($Z = -5.4404$, $p < 0.0001$, $n = 48$, Exact

General Independence Test). We did not detect any Tn5042 loss. For *P. putida*, Tn5042 tended to insert in a ~10 kb region near the origin of replication, while Tn5042 tended to insert in *P. fluorescens* near or inside Tn6291 (detected in 4/12 populations under mercury selection), in three cases representing the *de novo* formation of a composite resistance transposon. Here, Tn5042 became part of the cargo of Tn6291, broadening opportunities for spread, because subsequent events favouring Tn6291 mobilisation (perhaps different to those of Tn5042) will cause co-mobilisation of Tn5042 and its Hg^R genes. The pervasive mobility of Tn5042 supports a model in which TEs exploit plasmids to rapidly spread in the natural environment [5], consistent with sequence analysis suggesting Tn5042 was acquired relatively recently by pQBR57 (ref. 16) .

Surprisingly, we found plasmid size generally increased, primarily due to TE accumulation. Plasmid size in one clone evolved in 0 μg/g Hg(II) increased by over 10% compared with the ancestor (Figure 1). Increased plasmid size is expected to contribute to increased cost of plasmid carriage [27], however these results suggest such costs are negligibly small, and may be outweighed by transposition rates and/or general plasmid cost amelioration [26].

Discussion:

The central role of HGT in adaptation is increasingly apparent, as ever-wider sequencing of isolates reveals the dynamic nature of microbial genomes [4,5,28]. Between-species transfer of chromosomal genes occurred only where plasmid-encoded mercury resistance was not under positive selection and the plasmid persisted instead as an infectious

element. Bacterial genome evolution is determined by the interaction between selection and recombination [29] — here, we observed that recombination indeed makes an increased contribution to genome evolution when selection is relaxed. The transferred genes were part of the 'accessory' genome, which can vary even between closely-related strains and is often more strongly associated with ecological niche than phylogenetic lineage [30]. In this case, the transferred genes were located on TEs and putative transposases could be identified. This is relevant because TEs can transfer between replicons at a high rate [31], providing an efficient platform for the movement of genes between chromosomes and conjugative elements [2,3]. Plasmids and TEs have a close — even symbiotic — relationship. TEs can comprise a substantial fraction of a plasmid genome [32,33] and where their genes are under positive selection they can boost the fitness of the plasmids that carry them due to genetic linkage. Similarly, unless they encode their own conjugative machinery, TEs must collaborate with elements such as conjugative plasmids to access new hosts [34]; indeed models suggest that conjugative plasmids are required for TE survival and spread [35]. Transposase activity can be affected by stress, for example nutritional deprivation or oxidative damage [36], and one intriguing possibility is that stresses caused by plasmid acquisition [37] could signal to a TE that a vehicle had arrived, triggering transposition and thus increasing rates of exchange from the chromosome to that plasmid. At least one Pseudomonad TE has been shown to increase activity following conjugation [38].

Our results provide rare direct experimental evidence of pervasive plasmid-mediated gene mobilization, transfer and acquisition in a simple soil microcosm community. This

has profound implications for the spread of accessory genes in natural communities. Consistent with our findings, two recent studies of resistance plasmids in hospital outbreaks [5,39] indicate that TE mobilisation dominates plasmid evolution. Furthermore, both studies suggest that plasmids may have acquired TEs outside of patients, i.e. in the environment, where they are less likely to experience direct antibiotic selection. HGT vastly expands the evolutionary opportunities available to bacteria, allowing species to draw upon a collective mobile gene pool: our data indicate that environmental and ecological factors will be key modulators of the rate and extent of HGT in natural communities. HGT, particularly of antibiotic resistance and virulence genes, poses a major health concern [40] and understanding the ecology of HGT-mediated bacterial evolution will be crucial to predicting and designing interventions to prevent and mitigate such threats.

Materials and methods:

Experimental design

The evolution experiment, described previously [17], was designed to understand the effect of an alternative host species on plasmid population dynamics and evolution. The experiment used *Pseudomonas fluorescens* SBW25 and *P. putida* KT2440 — representative soil *Pseudomonas* species, a widespread and naturally co-occurring genus [41] — and the 307-kb Hg^R plasmid pQBR57 which was isolated from the same geographic site as *P. fluorescens* SBW25 (ref. 16). Cultures were grown at 28°C and 80% relative humidity in soil microcosms consisting of 10 g twice-autoclaved John Innes No. 2 potting soil, supplemented with 900 µl sterile H_2O or 900 µl $HgCl_2$ solution. We used a fully-

factoral design with two levels of mercury treatment (0 μg/g, or 16 μg/g); two levels of plasmid treatment (pQBR57+ starting with pQBR57-bearers at 50% frequency, or plasmid-free starting without plasmid); and three levels of culture treatment (single-species *P. fluorescens*, single-species *P. putida*, or co-culture with each species starting at 50% frequency). Six independent biological replicates ('populations') were initiated for each treatment, consistent with previous evolution experiments [42,43] and sufficient to detect differences in population dynamics between the treatments [17]. Each replicate was initiated from independent single colonies, and populations were blocked by replicate to minimize confounding effects. The experiment was not blinded. To control for marker effects, replicates a–c used gentamicin-labelled (Gm^R) *P. fluorescens* and streptomycin-labelled (Sm^R) *P. putida*, whereas replicates d–f used Sm^R *P. fluorescens* and Gm^R *P. putida*. Samples of culture (100 μl soil wash) were serially transferred into fresh soil microcosms containing either H_2O or $HgCl_2$ every four days for 65 transfers (estimated as ~440 generations [17]); this was decided before the experiment to be broadly consistent with other plasmid experimental evolution studies [42,43]. At 16 points during and at the end of the experiment samples were spread on selective media to isolate clones, which were archived for subsequent analysis. After 65 transfers, a random number generator was used to select one plasmid-bearing one plasmid-free clone (where present) from each pQBR57+ population for DNA sequencing. If plasmid-free or plasmid-bearing clones were present throughout the experiment but not at transfer 65, a clone from transfer 59 were used (this was the case for plasmid-bearing *P. putida* from single-species 16 μg/g replicate f, and plasmid-bearing *P. putida* from co-cultured 16 μg/g replicates a and c). We also sequenced ancestral clones, and three (single-species) or two (co-culture) clones

from plasmid-free treatments to test for mutations occurring in the absence of plasmid. No Tn6291, Tn4652, or Tn6290 activity was detected in the plasmid-free treatments.

DNA sequencing and analysis

DNA was extracted using the QIAGEN DNeasy kit, prepared using the TruSeq Nano DNA Library Preparation Kit (350 bp insert size), and sequenced on the Illumina HiSeq platform. Reads trimmed using Cutadapt (version 1.2.1) and Sickle (1.200) were analysed using the Bacterial and Archaeal Genome Analyser pipeline [19], which uses the bwa short read aligner [44] and calls variants using the Genome Analysis Toolkit HaplotypeCaller [45]. To identify structural variation (deletions, duplications and TE insertions) in the re-sequenced clones we used the BAGA module Structure, which uses a threshold ratio of non-proper to proper paired reads to identify putative genome disruptions. Reads mapping to putative disruptions were re-assembled using SPAdes [46], and contigs were aligned with the reference to identify structural variants. We also used two complementary approaches to identify structural variation: Breakdancer [47], and custom scripts that examined coverage for characteristic direct repeats introduced by TE insertion (increase in coverage of ≥25% over a <30 bp region, compared with neighbouring positions). These different approaches were broadly consistent, and all putative structural variants were examined using the Integrated Genome Viewer (IGV) [48]. Because of differences between ancestral clones and the sequenced reference genome, variation appearing in all samples (including the ancestor) was removed from the analysis. In addition, apparent variation in hard-to-map regions (identified in an examination of parallel mutations in IGV) was considered unreliable and excluded (Supplementary Table

3). We also examined putative SNV called near TE insertions, and removed these manually if miscalled. Representative TE insertions were tested by PCR on clones, and in all cases yielded products of the anticipated size.

Sequence analysis of ancestral clones revealed that in three cases, pQBR57 had acquired a TE before the experiment was initiated, indicated by dotted lines in Figure 1. In the Sm^R *P. fluorescens* ancestor, pQBR57 had acquired Tn6290 at position 164349–164354. This event likely occurred in *P. putida* UWC1 during preparation as a donor for transfer of pQBR57 into *P. fluorescens* [16] because Tn6290 is present in *P. putida* UWC1 and not in *P. fluorescens* SBW25. In Sm^R *P. putida*, pQBR57 had acquired Tn4652 at 152552–152558 while in Gm^R *P. putida* pQBR57 had acquired Tn4652 at 162797–162802. These events may have either occurred in the donor *P. putida* UWC1 strain or in the recipient *P. putida* KT2440 strain, as both contain identical copies of Tn4652. In any case, TE insertion must have occurred rapidly as our stocks were all prepared from single colonies and ancestral pQBR57 contains neither Tn6290 nor Tn4652 (ref. 16) Tn4652 insertion into resident plasmids is consistent with previous work which found Tn4652 in pQBR plasmids pQBR55 and pQBR44 (ref. 16) presumably after acquisition by *P. putida* UWC1 (ref. 49).

PCR analysis of clones

We tested archived clones for TE insertions by PCR. Standard reactions were performed using GoTaq Green Master Mix (Promega), 0.4 μM each primer (Supplementary Table 4), and 0.2 μl archived culture on a program of 95°C for 5 minutes, followed by 30 cycles

of 95°C for 30 seconds, 58°C for 30 seconds and 72°C for 1 minute, followed by a final extension of 72°C for 5 minutes. Tn6291 was detected in reisolated *P. putida* clones, and parallel reactions using primers targeting the *P. fluorescens* 16S rDNA locus were performed to rule out the presence of contaminating *P. fluorescens*.

Statistical analyses

To analyse the number of Tn5042 insertions in 0 µg/g and 16 µg/g Hg(II) we used the R package 'coin' to perform an Exact General Independence Test. To avoid pseudoreplication with populations from which >1 sample was sequenced, we analysed the mean number of Tn5042 insertions per species per population. To analyse the effect of mercury on conjugation dynamics we performed an Exact General Independence Test on plasmid distribution between donor and recipient after 5 transfers.

Data availability

Short read data is available at the European Nucleotide, Archive Project Accession PRJEB15009. Data presented in Figures 1–3 are on Dryad Digital Archive doi:10.5061/dryad.6gf28.

Code availability

The Bacterial and Archaeal Genome Analyser (BAGA) is available online at https://github.com/daveuu/baga . Representative scripts used to analyse our data are on Dryad Digital Archive doi:10.5061/dryad.6gf28.

References:

1. Halary, S., Leigh, J. W., Cheaib, B., Lopez, P. & Bapteste, E. Network analyses structure genetic diversity in independent genetic worlds. *Proceedings of the National Academy of Sciences* **107,** 127–132 (2010).
2. Norman, A., Hansen, L. H. & Sorensen, S. J. Conjugative plasmids: vessels of the communal gene pool. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364,** 2275–2289 (2009).
3. Frost, L. S., Leplae, R., Summers, A. O. & Toussaint, A. Mobile genetic elements: the agents of open source evolution. *Nat Rev Micro* **3,** 722–732 (2005).
4. Conlan, S. *et al*. Plasmid Dynamics in KPC-Positive Klebsiella pneumoniae during Long-Term Patient Colonization. *mBio* **7,** e00742–16 (2016).
5. Sheppard, A. E. *et al*. Nested Russian Doll-Like Genetic Mobility Drives Rapid Dissemination of the Carbapenem Resistance Gene blaKPC. *Antimicrobial Agents and Chemotherapy* **60,** 3767–3778 (2016).
6. Rasmussen, S. *et al*. Early Divergent Strains of Yersinia pestis in Eurasia 5,000 Years Ago. *Cell* **163,** 571–582 (2015).
7. Johnson, T. J. & Nolan, L. K. Pathogenomics of the virulence plasmids of Escherichia coli. *Microbiol*. *Mol*. *Biol*. *Rev*. **73,** 750–774 (2009).
8. Stoodley, P., Sauer, K., Davies, D. G. & Costerton, J. W. Biofilms as Complex Differentiated Communities. *Annu*. *Rev*. *Microbiol*. **56,** 187–209 (2002).
9. Lopatkin, A. J. *et al*. Antibiotics as a selective driver for conjugation dynamics. *Nature Microbiology* **1,** 16044 (2016).
10. Stevenson, C., Hall, J. P., Harrison, E., Wood, A. J. & Brockhurst, M. A. Gene

mobility promotes the spread of resistance in bacterial populations. *The ISME Journal* **63,** 1577 (2017).

11. Jacoby, G. A., Rogers, J. E., Jacob, A. E. & Hedges, R. W. Transposition of Pseudomonas toluene-degrading genes and expression in Escherichia coli. *Nature* **274,** 179–180 (1978).

12. Hedges, R. W. & Jacob, A. E. In vivo translocation of genes of Pseudomonas aeruginosa onto a promiscuously transmissible plasmid. *FEMS Microbiology Letters* **2,** 15–19 (1977).

13. Hemme, C. L. *et al*. Lateral Gene Transfer in a Heavy Metal-Contaminated-Groundwater Microbial Community. *mBio* **7,** e02234–15 (2016).

14. Xue, H. *et al*. Eco-Evolutionary Dynamics of Episomes among Ecologically Cohesive Bacterial Populations. *mBio* **6,** e00552–15 (2015).

15. Gomez, P. & Buckling, A. Bacteria-Phage Antagonistic Coevolution in Soil. *Science* **332,** 106–109 (2011).

16. Hall, J. P. J. *et al*. Environmentally co-occurring mercury resistance plasmids are genetically and phenotypically diverse and confer variable context-dependent fitness effects. *Environmental Microbiology* **17,** 5008–5022 (2015).

17. Hall, J. P. J., Wood, A. J., Harrison, E. & Brockhurst, M. A. Source–sink plasmid transfer dynamics maintain gene mobility in soil bacterial communities. *Proc*. *Natl*. *Acad*. *Sci*. *U*.*S*.*A*. **113,** 8260–8265 (2016).

18. Li, P., Feng, X. B., Qiu, G. L., Shang, L. H. & Li, Z. G. Mercury pollution in Asia: A review of the contaminated sites. *Journal of Hazardous Materials* **168,** 591–601 (2009).

19. Williams, D., Paterson, S., Brockhurst, M. A. & Winstanley, C. Refined analyses suggest that recombination is a minor source of genomic diversity in Pseudomonas aeruginosa chronic cystic fibrosis infections. *Microbial Genomics* **2,** (2016).
20. Silby, M. W. *et al*. Genomic and genetic analyses of diversity and plant interactions of Pseudomonas fluorescens. *Genome Biol* **10,** R51 (2009).
21. Ravindran, A., Jalan, N., Yuan, J. S., Wang, N. & Gross, D. C. Comparative genomics of Pseudomonas syringae pv. syringae strains B301D and HS191 and insights into intrapathovar traits associated with plant pathogenesis. *Microbiologyopen* **4,** 553–573 (2015).
22. Kivistik, P. A., Kivisaar, M. & Horak, R. Target Site Selection of Pseudomonas putida Transposon Tn4652. *Journal of Bacteriology* **189,** 3918–3921 (2007).
23. Levin, B. R., Stewart, F. M. & Rice, V. A. The kinetics of conjugative plasmid transmission: fit of a simple mass action model. *Plasmid* **2,** 247–260 (1979).
24. Hausner, M. & Wuertz, S. High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. *Applied and Environmental Microbiology* **65,** 3710–3713 (1999).
25. Bergstrom, C. T., Lipsitch, M. & Levin, B. R. Natural selection, infectious transfer and the existence conditions for bacterial plasmids. *Genetics* **155,** 1505–1519 (2000).
26. Harrison, E. *et al*. Rapid compensatory evolution promotes the survival of conjugative plasmids. *Mob Genet Elements* **6,** e1179074–7 (2016).
27. Baltrus, D. A. Exploring the costs of horizontal gene transfer. *Trends in Ecology & Evolution* **28,** 489–495 (2013).

28. McCarthy, A. J. *et al*. Extensive horizontal gene transfer during Staphylococcus aureus co-colonization in vivo. *Genome Biol Evol* **6,** 2697–2708 (2014).
29. Shapiro, B. J. How clonal are bacteria over time? *Current Opinion in Microbiology* **31,** 116–123 (2016).
30. Polz, M. F., Alm, E. J. & Hanage, W. P. Horizontal gene transfer and the evolution of bacterial and archaeal population structure. *Trends Genet*. **29,** 170–175 (2013).
31. Sousa, A., Bourgard, C., Wahl, L. M. & Gordo, I. Rates of transposition in Escherichia coli. *Biology Letters* **9,** 20130838–20130838 (2013).
32. Dennis, J. J. The evolution of IncP catabolic plasmids. *Current Opinion in Biotechnology* **16,** 291–298 (2005).
33. Revilla, C. *et al*. Different pathways to acquiring resistance genes illustrated by the recent evolution of IncW plasmids. *Antimicrobial Agents and Chemotherapy* **52,** 1472–1480 (2008).
34. Carraro, N., Rivard, N., Burrus, V. & Ceccarelli, D. Mobilizable genomic islands, different strategies for the dissemination of multidrug resistance and other adaptive traits. *Mob Genet Elements* **7,** 1–6 (2017).
35. Condit, R., Stewart, F. M. & Levin, B. R. The population biology of bacterial transposons: a priori conditions for maintenance as parasitic DNA. *Am Nat* **132,** 129–147 (1988).
36. Twiss, E., Coros, A. M., Tavakoli, N. P. & Derbyshire, K. M. Transposition is modulated by a diverse set of host factors in Escherichia coliand is stimulated by nutritional stress. *Mol*. *Microbiol*. **57,** 1593–1607 (2005).
37. Baharoglu, Z., Bikard, D. & Mazel, D. Conjugative DNA Transfer Induces the

Bacterial SOS Response and Promotes Antibiotic Resistance Development through Integron Activation. *PLoS Genet* **6,** e1001165–10 (2010).

38. Christie-Oleza, J. A., Lanfranconi, M. P., Nogales, B., Lalucat, J. & Bosch, R. Conjugative interaction induces transposition of ISPst9 in Pseudomonas stutzeri AN10. *Journal of Bacteriology* **191,** 1239–1247 (2009).

39. He, S. *et al*. Mechanisms of Evolution in High-Consequence Drug Resistance Plasmids. *mBio* **7,** e01987–16–11 (2016).

40. O'Neill, J. *Tackling drug-resistant infections globally: final report and recommendations*. (London: Wellcome Trust & HM Government, 2016).

41. Cho, J. C. & Tiedje, J. M. Biogeography and degree of endemicity of fluorescent Pseudomonas strains in soil. *Applied and Environmental Microbiology* **66,** 5448–5456 (2000).

42. De Gelder, L., Williams, J. J., Ponciano, J. M., Sota, M. & Top, E. M. Adaptive Plasmid Evolution Results in Host-Range Expansion of a Broad-Host-Range Plasmid. *Genetics* **178,** 2179–2190 (2008).

43. Harrison, E., Guymer, D., Spiers, A. J., Paterson, S. & Brockhurst, M. A. Parallel compensatory evolution stabilizes plasmids across the parasitism-mutualism continuum. *Curr*. *Biol*. **25,** 2034–2039 (2015).

44. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25,** 1754–1760 (2009).

45. McKenna, A. *et al*. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. **20,** 1297–1303 (2010).

46. Bankevich, A. *et al*. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology* **19,** 455–477 (2012).

47. Chen, K. *et al*. BreakDancer: an algorithm for high-resolution mapping of genomic structural variation. *Nature Methods* **6,** 677–681 (2009).

48. Robinson, J. T. *et al*. Integrative genomics viewer. *Nature Biotechnology* **29,** 24–26 (2011).

49. Lilley, A. K., Bailey, M. J., Day, M. J. & Fry, J. C. Diversity of mercury resistance plasmids obtained by exogenous isolation from the bacteria of sugar beet in three successive years. *FEMS Microbiology Ecology* **20,** 211–227 (1996).

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Author contributions: JPJH, EH, and MAB designed the study; JPJH collected data; JPJH, DW and SP analysed the data. JPJH and MAB drafted the manuscript. All authors discussed results and commented on the manuscript.

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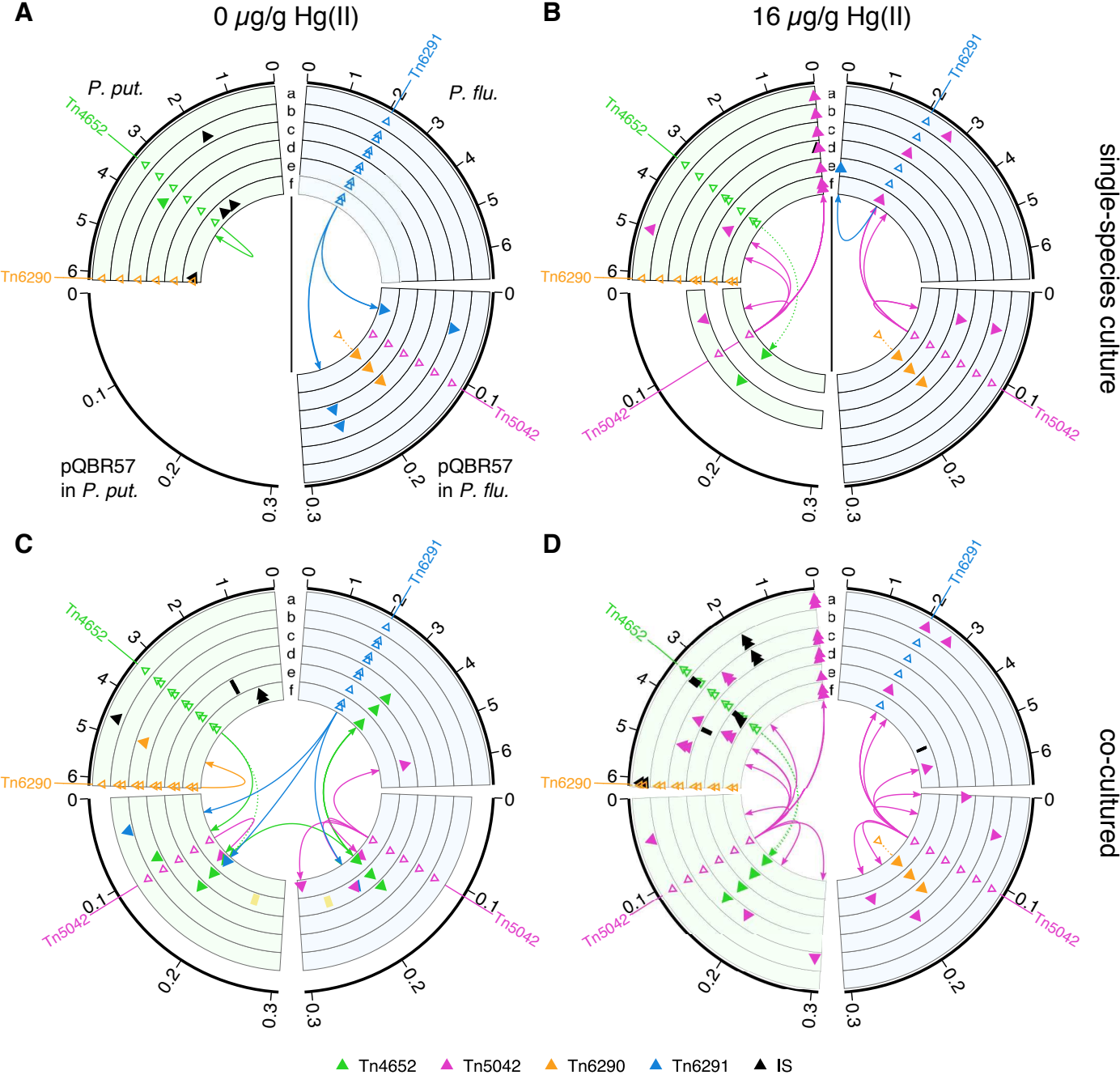
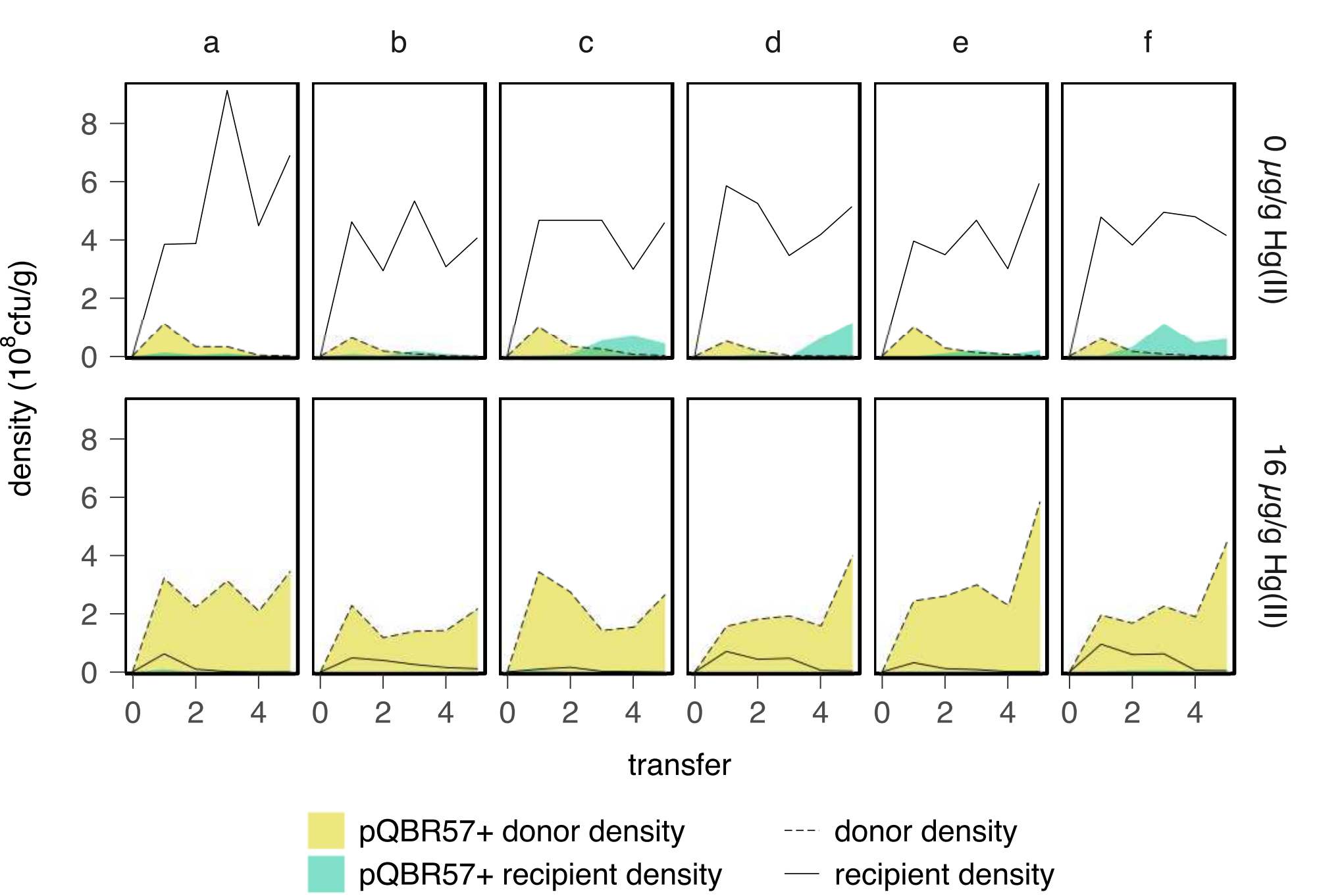
Figure Legends:

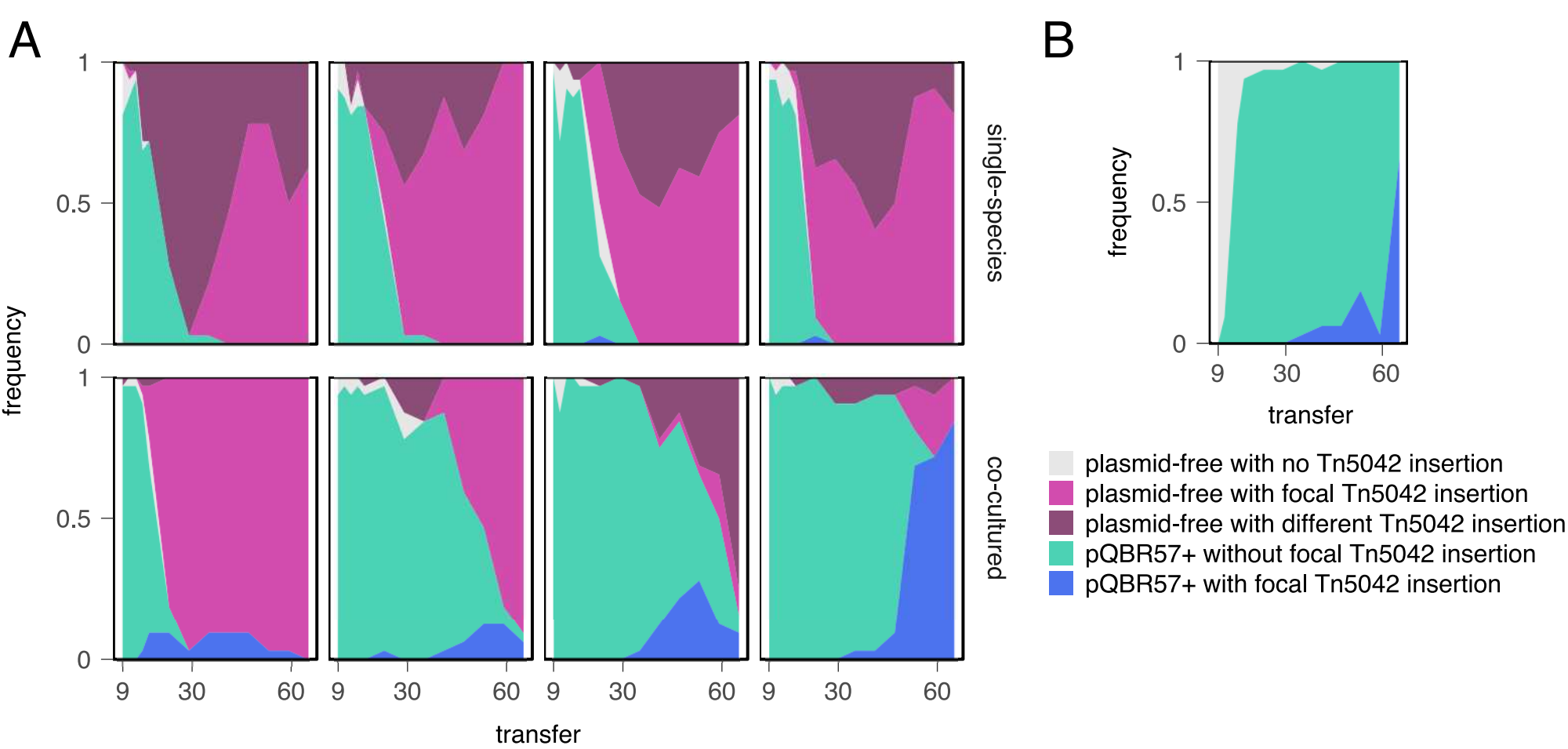
Figure 1. Evolved clones show extensive within- and between-species gene mobilisation. Each panel shows events detected in evolved *P*. *putida* (left, light green) and *P*. *fluorescens* (right, light blue), with changes in associated pQBR57 (if present) shown below. In each panel, six concentric lanes a–f indicate independent populations. One clone was sequenced from each population for each species, except where both plasmid-bearing and plasmid-free genotypes were detected. In this case, we sequenced one clone of each, with the plasmid-bearing clone indicated by the inner set of symbols in that lane (Supplementary Table 1). Duplicative insertions of large TEs are indicated by filled triangles coloured according to TE type (see key) and connected to ancestral positions (indicated by open triangles) by an arrow describing direction of duplication. Dotted lines indicate insertions that occurred before the evolution experiment was initiated (see Methods). Insertions of smaller insertion sequence (IS) elements are in black. Black bars indicate large deletions, and yellow bars (panel C, replicate e) indicate large tandem duplications. Scale is given in Mbp, and replicons are scaled to the same size for clarity. (A) Clones evolved in single-species populations with 0 μg/g Hg(II). (B) Clones evolved in single-species population with 16 μg/g Hg(II). In panels A and B, lines indicate the

458 physical separation of the two species. (C) Clones evolved in co-cultured populations
459 with 0 μg/g Hg(II). (D) Clones evolved in co-cultured populations with 16 μg/g Hg(II).
460
461 **Figure 2.**
462 Plasmid dynamics are altered under positive selection. Top row: plasmid-bearing
463 ('donor') and plasmid-free ('recipient') *P. fluorescens* were mixed in approximately
464 equal ratios and cultured for 5 transfers in 0 μg/g Hg(II) microcosms. Densities of donors
465 (dotted line) and recipients (solid line) and their plasmid statuses (donor, yellow fill;
466 recipient, green fill; filled areas are overlaid) were estimated each transfer by plating onto
467 selective media and replica plating onto Hg(II) where appropriate. Each panel represents
468 an independent population. Bottom row: as top row except with 16 μg/g Hg(II).
469 Conjugation makes a reduced contribution to plasmid dynamics under 16 μg/g Hg(II) (Z
470 = 2.88, $p = 0.002$, $n = 12$, Exact General Independence Test). These results are similar to
471 those reported by Stevenson et al.[10], showing that this pattern holds in soil microcosms.
472
473 **Figure 3.** Spread of chromosomally-acquired mercury resistance. (A) Frequency
474 dynamics of focal Tn5042 insertions in *P. putida* chromosomes under 16 μg/g Hg(II)
475 were tracked from transfer 9 (when insertions were first detected) to transfer 65. For each
476 timepoint in each population, presence and frequency of the focal insertion was tested by
477 PCR on ~30 clones using primers bridging the transposon and the chromosome (this
478 giving a 95% chance of detecting a subpopulation comprising 10% of the total); other
479 Tn5042 insertions were identified previously as pQBR57– merA+ clones [17]. Plasmid-
480 bearing clones were identified previously [17]. Frequencies of different genotypes are

indicated by filled stacked areas. Each panel represents an independent separate population: top row, single-species populations a, b, c, e; bottom row, co-cultured with *P. fluorescens* populations c, d, e, f. (B) Frequency dynamics of focal Tn5042 insertion in *P. fluorescens* chromosome under 0 μg/g Hg(II). This population was co-cultured with *P. putida* (replicate b).


A
0 μg/g Hg(II)
B
16 μg/g Hg(II)
single-species culture
C
D
co-cultured
P. put.
P. flu.
pQBR57 in P. put.
pQBR57 in P. flu.
Tn4652
Tn6291
Tn6290
Tn5042
a
b
c
d
e
f
0
1
2
3
4
5
6
0.1
0.2
0.3
Tn4652
Tn5042
Tn6290
Tn6291
IS


a
b
c
d
e
f
0 μg/g Hg(II)
16 μg/g Hg(II)
density (10^8cfu/g)
0
2
4
6
8
transfer
pQBR57+ donor density
pQBR57+ recipient density
donor density
recipient density


A
B
frequency
transfer
single-species
co-cultured
plasmid-free with no Tn5042 insertion
plasmid-free with focal Tn5042 insertion
plasmid-free with different Tn5042 insertion
pQBR57+ without focal Tn5042 insertion
pQBR57+ with focal Tn5042 insertion