

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

3
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14
15 **Main text:**

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

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

30
31 Conjugative plasmids — semi-autonomous mobile genetic elements that transfer between
32 bacteria — are key agents of horizontal gene transfer (HGT) ^{1,2}, facilitating rapid
33 bacterial adaptation by spreading ecologically important traits between lineages ³. The
34 physical movement or duplication of genes (gene mobilisation) between chromosomes
35 and plasmids, and their subsequent transfer between hosts, can be decisive in microbial
36 evolution, and has facilitated acquisition of antimicrobial resistance ^{4,5} and emergence of
37 virulent pathogens ^{6,7}. Such mobilisation can be facilitated by transposable elements
38 (TEs), which encode enzymes (transposases) allowing transfer of genes between
39 replicons ². However, the effects of ecological factors and natural selection on gene
40 mobilisation and subsequent HGT is unclear. This is particularly the case for natural
41 environments with a high degree of spatial structure ⁸, which is rarely captured by
42 experimental studies. An outstanding question is how positive selection for plasmid-
43 encoded traits, like resistance genes, affects the ability of that plasmid to spread genes
44 through a community. Although positive selection can favour HGT by benefitting
45 bacteria which have acquired the plasmid (transconjugants), it can also prevent HGT by
46 killing or inhibiting growth of potential plasmid recipients ^{9,10}.

47
48 Experimental evolution of bacterial communities is a powerful tool for exploring the
49 evolutionary dynamics of plasmid-mediated HGT, bridging the gap between simplified
50 short-term laboratory studies^{11,12} and comparative genomics of natural populations
51^{4,5,13,14}. To investigate how positive selection for plasmid-borne genes and the presence of
52 an alternative host species interact to determine plasmid dynamics, we established
53 communities of the common soil bacteria *Pseudomonas fluorescens* and *Pseudomonas*
54 *putida*, either alone or in co-culture, in sterilized soil microcosms, which offer a complex,
55 spatially-structured, and experimentally tractable environment¹⁵⁻¹⁷. Communities began
56 with the 307-kb conjugative plasmid pQBR57 at ~50% frequency; this plasmid was
57 isolated from agricultural soil and carries a 7-kb mercury resistance (Hg^R) TE, Tn5042.
58 We also established control communities without pQBR57. Microcosms were
59 supplemented with mercuric chloride to 16 $\mu\text{g/g}$ Hg(II), or an equivalent volume of
60 water (0 $\mu\text{g/g}$ Hg(II)). This level of mercury contamination, similar to that found in
61 industrial or post-industrial sites¹⁸, selects for specific Hg^R but does not necessitate it¹⁶.
62 Six replicate populations for each combination of treatments were grown for ~440
63 generations. Analysis of plasmid frequency dynamics showed that pQBR57 was
64 generally maintained by *P. fluorescens* and lost by *P. putida*, but persisted in *P. putida*
65 when co-cultured with *P. fluorescens*, due to interspecific ‘source-sink’ plasmid transfer
66¹⁷. To investigate consequent effects on gene mobilisation and transfer we sequenced
67 clones from the beginning and the end of the experiment, and used the Bacterial and
68 Archaeal Genome Analyser (BAGA) pipeline to identify structural variations¹⁹.
69

70 **Results:**

71 We detected multiple, independent gene mobilisation events between plasmid and
72 chromosome in each species. Strikingly and unexpectedly, we also identified numerous
73 interspecific transfers of chromosomal genes in the co-cultured treatments, facilitated by
74 pQBR57 (Fig. 1). Gene transfer from *P. fluorescens* to *P. putida* was exemplified by a
75 previously-unannotated *P. fluorescens* TE, Tn6291, in *P. putida* plasmids from 2/6 co-
76 cultured communities (Fig. 1, replicates b and f). Subsequent PCR analyses found
77 Tn6291 in *P. putida* clones from 2 further co-cultured communities (replicates d and e).
78 Tn6291, a 22 kb TE carrying 25 predicted open reading frames and located between
79 2060105 and 2082440 in the *P. fluorescens* SBW25 reference sequence (ENA identifier
80 AM181176, part of genomic island SBW_GI-1 (ref. 20)), carries an array of cargo genes
81 with putative cytochrome c/d oxidase functions. The presence of Tn6291 in *P. putida*
82 indicates these genes mobilised from the *P. fluorescens* chromosome onto pQBR57, and
83 subsequently transferred between species via conjugation. All Tn6291+ *P. putida* clones
84 were also positive for pQBR57, suggesting Tn6291 remains plasmid-borne in these
85 isolates. Indeed, we detected plasmid-borne Tn6291 in 4/6 0 µg/g Hg(II) single-species
86 *P. fluorescens* populations, and BLAST analysis shows a similar transposon in another
87 soil Pseudomonad, *P. syringae* pv. *syringae* B301D (ref. 21), suggesting Tn6291
88 mobilises readily.

89

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

93 kb TE closely related to the Tn4651 toluene degradation transposon and encodes various
94 putative enzymes including a diacylglycerol kinase and a sulfatase. Tn4652 mobilisation
95 to the plasmid occurred readily, with events already detectible in the ancestral *P. putida*
96 clones used to inoculate the soil and begin the experiment (see Materials and Methods).
97 PCR analysis of clones obtained over the course of the evolution experiment detected
98 Tn4652+ plasmids in *P. fluorescens* as early as transfer 3, however Tn4652 insertion in
99 the *P. fluorescens* chromosome was only detected later, after transfer 41 (Supplementary
100 Table 2). In all cases, Tn4652 inserted in a region of the *P. fluorescens* chromosome with
101 atypical sequence composition, likely to be recently acquired DNA ²⁰.
102
103 Importantly, interspecific transfer of chromosomal TEs via the plasmid was only detected
104 in populations grown without positive selection for the plasmid (0 µg/g Hg(II)). The
105 amount of plasmid conjugation occurring, and thus opportunities for interspecific gene
106 transfer, is likely a function of the densities of plasmid bearers and recipients ^{23,24}. By
107 killing potential plasmid recipients, mercury selection reduces encounters between
108 plasmid donors and recipients, and therefore conjugation ⁹. Indeed, short-term
109 experiments examining pQBR57 transfer (Figure 2) showed reduced effects of
110 conjugation on plasmid dynamics when the plasmid was under selection ¹⁰, implying
111 limited gene exchange. Together, these data suggest that positive selection for plasmid-
112 borne resistance genes reduced the ability of that plasmid to facilitate HGT of
113 chromosomal genes.

114

115 While mercury selection reduced TE transfer between species, we detected frequent
116 mobilisation of the Hg^R TE Tn5042 from pQBR57 to the chromosome. Single-species *P.*
117 *putida* populations tended to lose the plasmid ¹⁷, and sequences show that under mercury
118 selection this was facilitated by acquisition of chromosomal Tn5042. To track the
119 acquisition of chromosomal Tn5042 by *P. putida* populations, we designed PCR primers
120 targeting ‘focal’ Tn5042 insertions (i.e. insertions detected in the end-point genome
121 sequences) in eight different *P. putida* populations, and applied these to clones collected
122 across the experiment. As with single-species *P. putida* populations, chromosomal
123 Tn5042 was readily acquired by co-cultured *P. putida*, although these populations tended
124 to also maintain pQBR57 (Figure 3). Similarly, chromosomal Tn5042 was detected in the
125 *P. fluorescens* chromosome, which maintained the plasmid, despite its redundancy ¹⁷.
126 These findings suggest that long-term plasmid maintenance largely depends on
127 community context and on compatibility between plasmid and host, and, provided there
128 are no restrictions on recombination of plasmid genes into the chromosome, is unlikely to
129 be secured by positive selection for accessory genes alone ^{17,25,26}.
130
131 Tn5042 also mobilised in the mercury-free treatments: we detected three instances of
132 Tn5042 multiplying on plasmids, and one instance of Tn5042 copying to the *P.*
133 *fluorescens* chromosome (which occurred by transfer 35, see Figure 3). Tn5042 insertions
134 sometimes occurred multiple times in a lineage — in co-cultured *P. putida* with 16 µg/g
135 Hg(II), one clone (from replicate c) ultimately carried six copies. Although Tn5042 copy
136 number increased in some clones from the mercury-free treatment, we detected more
137 copies in clones evolved under mercury selection ($Z = -5.4404$, $p < 0.0001$, $n = 48$, Exact

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

148

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

155

156 **Discussion:**

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

161 element. Bacterial genome evolution is determined by the interaction between selection
162 and recombination²⁹ — here, we observed that recombination indeed makes an increased
163 contribution to genome evolution when selection is relaxed. The transferred genes were
164 part of the ‘accessory’ genome, which can vary even between closely-related strains and
165 is often more strongly associated with ecological niche than phylogenetic lineage³⁰. In
166 this case, the transferred genes were located on TEs and putative transposases could be
167 identified. This is relevant because TEs can transfer between replicons at a high rate³¹,
168 providing an efficient platform for the movement of genes between chromosomes and
169 conjugative elements^{2,3}. Plasmids and TEs have a close — even symbiotic —
170 relationship. TEs can comprise a substantial fraction of a plasmid genome^{32,33} and where
171 their genes are under positive selection they can boost the fitness of the plasmids that
172 carry them due to genetic linkage. Similarly, unless they encode their own conjugative
173 machinery, TEs must collaborate with elements such as conjugative plasmids to access
174 new hosts³⁴; indeed models suggest that conjugative plasmids are required for TE
175 survival and spread³⁵. Transposase activity can be affected by stress, for example
176 nutritional deprivation or oxidative damage³⁶, and one intriguing possibility is that
177 stresses caused by plasmid acquisition³⁷ could signal to a TE that a vehicle had arrived,
178 triggering transposition and thus increasing rates of exchange from the chromosome to
179 that plasmid. At least one Pseudomonad TE has been shown to increase activity
180 following conjugation³⁸.

181

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

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

196

197 **Materials and methods:**

198 Experimental design

199 The evolution experiment, described previously ¹⁷, was designed to understand the effect
200 of an alternative host species on plasmid population dynamics and evolution. The
201 experiment used *Pseudomonas fluorescens* SBW25 and *P. putida* KT2440 —
202 representative soil *Pseudomonas* species, a widespread and naturally co-occurring genus
203 ⁴¹ — and the 307-kb Hg^R plasmid pQBR57 which was isolated from the same geographic
204 site as *P. fluorescens* SBW25 (ref. 16). Cultures were grown at 28°C and 80% relative
205 humidity in soil microcosms consisting of 10 g twice-autoclaved John Innes No. 2 potting
206 soil, supplemented with 900 µl sterile H₂O or 900 µl HgCl₂ solution. We used a fully-

207 factorial design with two levels of mercury treatment (0 µg/g, or 16 µg/g); two levels of
208 plasmid treatment (pQBR57+ starting with pQBR57-bearers at 50% frequency, or
209 plasmid-free starting without plasmid); and three levels of culture treatment (single-
210 species *P. fluorescens*, single-species *P. putida*, or co-culture with each species starting at
211 50% frequency). Six independent biological replicates (‘populations’) were initiated for
212 each treatment, consistent with previous evolution experiments^{42,43} and sufficient to
213 detect differences in population dynamics between the treatments¹⁷. Each replicate was
214 initiated from independent single colonies, and populations were blocked by replicate to
215 minimize confounding effects. The experiment was not blinded. To control for marker
216 effects, replicates a–c used gentamicin-labelled (Gm^R) *P. fluorescens* and streptomycin-
217 labelled (Sm^R) *P. putida*, whereas replicates d–f used Sm^R *P. fluorescens* and Gm^R *P.*
218 *putida*. Samples of culture (100 µl soil wash) were serially transferred into fresh soil
219 microcosms containing either H₂O or HgCl₂ every four days for 65 transfers (estimated as
220 ~440 generations¹⁷); this was decided before the experiment to be broadly consistent
221 with other plasmid experimental evolution studies^{42,43}. At 16 points during and at the end
222 of the experiment samples were spread on selective media to isolate clones, which were
223 archived for subsequent analysis. After 65 transfers, a random number generator was
224 used to select one plasmid-bearing one plasmid-free clone (where present) from each
225 pQBR57+ population for DNA sequencing. If plasmid-free or plasmid-bearing clones
226 were present throughout the experiment but not at transfer 65, a clone from transfer 59
227 were used (this was the case for plasmid-bearing *P. putida* from single-species 16 µg/g
228 replicate f, and plasmid-bearing *P. putida* from co-cultured 16 µg/g replicates a and c).
229 We also sequenced ancestral clones, and three (single-species) or two (co-culture) clones

230 from plasmid-free treatments to test for mutations occurring in the absence of plasmid.
231 No Tn6291, Tn4652, or Tn6290 activity was detected in the plasmid-free treatments.
232
233 DNA sequencing and analysis
234 DNA was extracted using the QIAGEN DNeasy kit, prepared using the TruSeq Nano
235 DNA Library Preparation Kit (350 bp insert size), and sequenced on the Illumina HiSeq
236 platform. Reads trimmed using Cutadapt (version 1.2.1) and Sickle (1.200) were analysed
237 using the Bacterial and Archaeal Genome Analyser pipeline ¹⁹, which uses the bwa short
238 read aligner ⁴⁴ and calls variants using the Genome Analysis Toolkit HaplotypeCaller ⁴⁵.
239 To identify structural variation (deletions, duplications and TE insertions) in the re-
240 sequenced clones we used the BAGA module Structure, which uses a threshold ratio of
241 non-proper to proper paired reads to identify putative genome disruptions. Reads
242 mapping to putative disruptions were re-assembled using SPAdes ⁴⁶, and contigs were
243 aligned with the reference to identify structural variants. We also used two
244 complementary approaches to identify structural variation: Breakdancer ⁴⁷, and custom
245 scripts that examined coverage for characteristic direct repeats introduced by TE insertion
246 (increase in coverage of $\geq 25\%$ over a < 30 bp region, compared with neighbouring
247 positions). These different approaches were broadly consistent, and all putative structural
248 variants were examined using the Integrated Genome Viewer (IGV) ⁴⁸. Because of
249 differences between ancestral clones and the sequenced reference genome, variation
250 appearing in all samples (including the ancestor) was removed from the analysis. In
251 addition, apparent variation in hard-to-map regions (identified in an examination of
252 parallel mutations in IGV) was considered unreliable and excluded (Supplementary Table

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

256

257 Sequence analysis of ancestral clones revealed that in three cases, pQBR57 had acquired
258 a TE before the experiment was initiated, indicated by dotted lines in Figure 1. In the
259 Sm^R *P. fluorescens* ancestor, pQBR57 had acquired Tn6290 at position 164349–164354.
260 This event likely occurred in *P. putida* UWC1 during preparation as a donor for transfer
261 of pQBR57 into *P. fluorescens*¹⁶ because Tn6290 is present in *P. putida* UWC1 and not
262 in *P. fluorescens* SBW25. In Sm^R *P. putida*, pQBR57 had acquired Tn4652 at 152552–
263 152558 while in Gm^R *P. putida* pQBR57 had acquired Tn4652 at 162797–162802. These
264 events may have either occurred in the donor *P. putida* UWC1 strain or in the recipient *P.*
265 *putida* KT2440 strain, as both contain identical copies of Tn4652. In any case, TE
266 insertion must have occurred rapidly as our stocks were all prepared from single colonies
267 and ancestral pQBR57 contains neither Tn6290 nor Tn4652 (ref. 16) Tn4652 insertion
268 into resident plasmids is consistent with previous work which found Tn4652 in pQBR
269 plasmids pQBR55 and pQBR44 (ref. 16) presumably after acquisition by *P. putida*
270 UWC1 (ref. 49).

271

272 PCR analysis of clones

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

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

280

281 Statistical analyses

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

288

289 Data availability

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

293

294 Code availability

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

298

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423 successive years. *FEMS Microbiology Ecology* **20**, 211–227 (1996).

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425

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430

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440 **Figure Legends:**

441

442 **Figure 1.** Evolved clones show extensive within- and between-species gene mobilisation.

443 Each panel shows events detected in evolved *P. putida* (left, light green) and *P.*

444 *fluorescens* (right, light blue), with changes in associated pQBR57 (if present) shown

445 below. In each panel, six concentric lanes a–f indicate independent populations. One

446 clone was sequenced from each population for each species, except where both plasmid-

447 bearing and plasmid-free genotypes were detected. In this case, we sequenced one clone

448 of each, with the plasmid-bearing clone indicated by the inner set of symbols in that lane

449 (Supplementary Table 1). Duplicative insertions of large TEs are indicated by filled

450 triangles coloured according to TE type (see key) and connected to ancestral positions

451 (indicated by open triangles) by an arrow describing direction of duplication. Dotted lines

452 indicate insertions that occurred before the evolution experiment was initiated (see

453 Methods). Insertions of smaller insertion sequence (IS) elements are in black. Black bars

454 indicate large deletions, and yellow bars (panel C, replicate e) indicate large tandem

455 duplications. Scale is given in Mbp, and replicons are scaled to the same size for clarity.

456 (A) Clones evolved in single-species populations with 0 $\mu\text{g/g}$ Hg(II). (B) Clones evolved

457 in single-species population with 16 $\mu\text{g/g}$ 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 $\mu\text{g/g}$ Hg(II). (D) Clones evolved in co-cultured populations with 16 $\mu\text{g/g}$ Hg(II).

460

461 **Figure 2.**

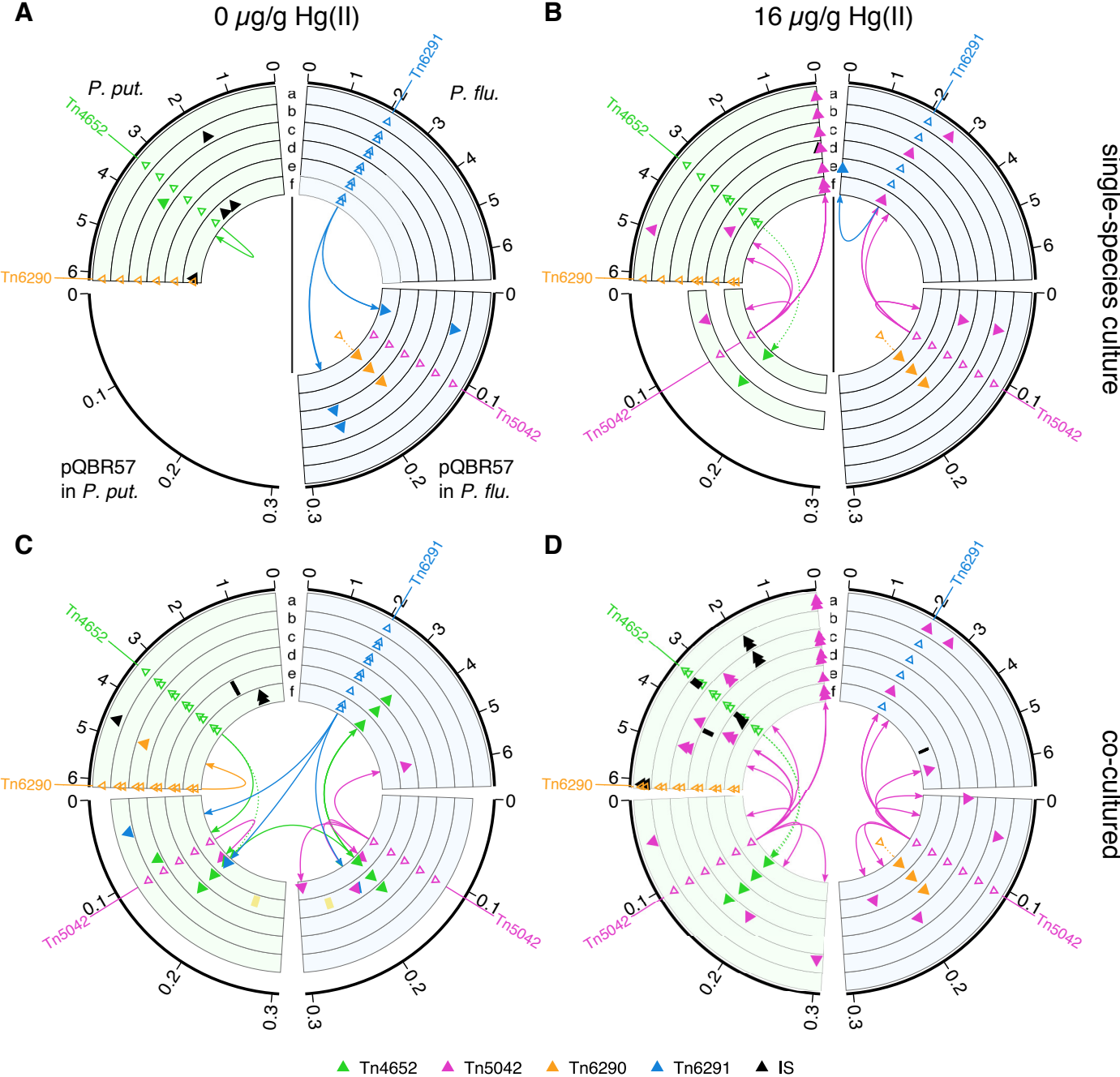
462 Plasmid dynamics are altered under positive selection. Top row: plasmid-bearing
463 ('donor') and plasmid-free ('recipient') *P. fluorescens* were mixed in approximately
464 equal ratios and cultured for 5 transfers in 0 $\mu\text{g/g}$ Hg(II) microcosms. Densities of donors
465 (dotted line) and 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 $\mu\text{g/g}$ Hg(II).

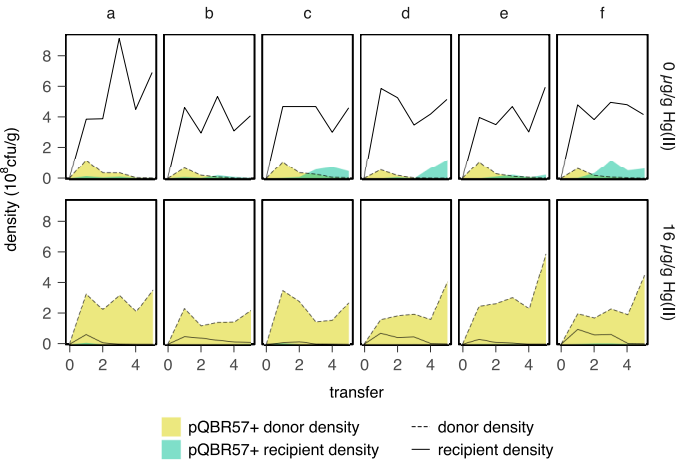
469 Conjugation makes a reduced contribution to plasmid dynamics under 16 $\mu\text{g/g}$ Hg(II) (Z
470 = 2.88, $p = 0.002$, $n = 12$, Exact General Independence Test). These results are similar to
471 those reported by Stevenson et al.¹⁰, showing that this pattern holds in soil microcosms.

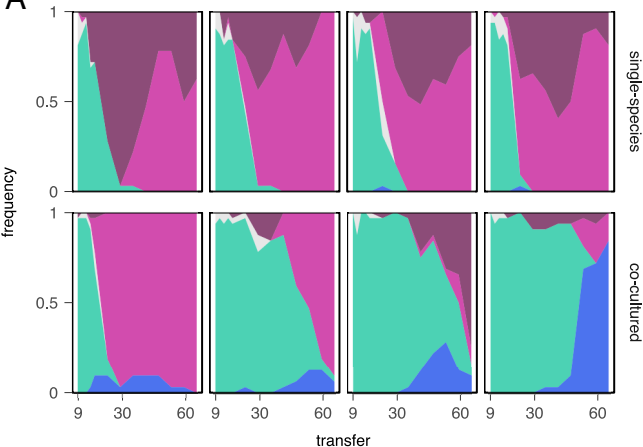
472

473 **Figure 3.** Spread of chromosomally-acquired mercury resistance. (A) Frequency
474 dynamics of focal Tn5042 insertions in *P. putida* chromosomes under 16 $\mu\text{g/g}$ 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+ clones¹⁷. Plasmid-
480 bearing clones were identified previously¹⁷. Frequencies of different genotypes are

481 indicated by filled stacked areas. Each panel represents an independent separate
482 population: top row, single-species populations a, b, c, e; bottom row, co-cultured with *P.*
483 *fluorescens* populations c, d, e, f. (B) Frequency dynamics of focal Tn5042 insertion in *P.*
484 *fluorescens* chromosome under 0 µg/g Hg(II). This population was co-cultured with *P.*
485 *putida* (replicate b).
486





A**B**