The proficiency of the original host species determines community-level plasmid dynamics

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ABSTRACT

Plasmids are common in natural bacterial communities, facilitating bacterial evolution via horizontal gene transfer. Bacterial species vary in their proficiency to host plasmids: Whereas plasmids are stably maintained in some species regardless of selection for plasmid-encoded genes, in other species, even beneficial plasmids are rapidly lost. It is, however, unclear how this variation in host proficiency affects plasmid persistence in communities. Here, we test this using multispecies bacterial soil communities comprising species varying in their proficiency to host a large conjugative mercury resistance plasmid, pQBR103. The plasmid reached higher community-level abundance where beneficial and when introduced to the community in a more proficient host species. Proficient plasmid host species were also better able to disseminate the plasmid to a wider diversity of host species. These findings suggest that the dynamics of plasmids in natural bacterial communities depend not only upon the plasmid's attributes and the selective environment, but also upon the proficiency of their host species.

52 **INTRODUCTION**

53 Mobile genetic elements (MGEs) like plasmids, temperate bacteriophages, and transposons, are
54 important agents of horizontal gene transfer (HGT) driving the diversification of bacterial genomes
55 (Frost *et al.* 2005; Hall, Brockhurst and Harrison 2017a; Brockhurst *et al*. 2019). Conjugative plasmids
56 contain genes encoding core plasmid functions – including their own propagation, replication, stability
57 and transfer – along with accessory genes that encode traits like antibiotic and metal resistance
58 (Norman, Hansen and Sørensen 2009). While the plasmid's accessory genes may directly benefit the
59 host cell by providing them with new ecological functions, plasmid acquisition can impose a heavy
60 fitness burden on the host cell (Baltrus 2013; San Millan and Maclean 2017). Mathematical models of
61 plasmid population dynamics suggest that the plasmid cost, conjugation rate, segregation rate, and the
62 strength of positive selection are key parameters determining plasmid survival in bacterial populations
63 (Stewart and Levin 1977; Levin, Stewart and Rice 1979; Simonsen *et al.* 1990; Bergstrom, Lipsitch and
64 Levin 2000).

65 Plasmids are expected to spread under positive selection for their encoded accessory genes (San
66 Millan *et al*. 2014; Harrison *et al*. 2015), however, because accessory genes can be captured by the
67 bacterial chromosome rendering the plasmid redundant, positive selection does not guarantee the long-
68 term survival of plasmids (Bergstrom, Lipsitch and Levin 2000). Meanwhile, in the absence of positive
69 selection, plasmids are expected to decline in frequency due to purifying selection because the benefits
70 of accessory genes do not outweigh the costs of plasmid carriage (Bergstrom, Lipsitch and Levin 2000).
71 Since rates of conjugation appear to often be too low for plasmids to persist as infectious elements
72 (although see: Lopatkin *et al*. (2017) and Stevenson *et al*. (2017)), it has been argued that the widespread
73 distribution of plasmids is paradoxical (the plasmid paradox: Harrison and Brockhurst (2012)). Yet,
74 plasmids have been found to stably persist in natural bacterial communities in the absence of measurable
75 positive selection, where the factors allowing plasmid stability are puzzling (Heuer and Smalla 2012).

76 Most studies of plasmid dynamics focus on a single-host species, whereas, in natural bacterial
77 communities, many potential host species co-exist, potentially broadening the range of conditions under
78 which plasmids can survive. This limitation of current understanding is particularly interesting

79 considering that several studies have shown that plasmids are not equally stable across host species (De
80 Gelder *et al.* 2007; Kottara *et al.* 2018; Sakuda *et al.* 2018). For example, while the mercury resistance
81 plasmid pQBR103 was highly stable for >400 generations with or without mercury selection in *P.*
82 *fluorescens* and *P. savastanoi*, it was unstable to varying degrees in *P. stutzeri* (generally lost within
83 ~100-400 generations), *P. aeruginosa* and *P. putida* (<6 generations) even with strong mercury
84 selection (Kottara *et al*. 2018). Here, we define host proficiency as the ability of a bacterial host species
85 to stably maintain a conjugative plasmid within its population.

86 Hall *et al*. (2016) showed, by tracking the dynamics of the mercury resistance plasmid pQBR57
87 in a two-species soil community of *P. fluorescens* and *P. putida*, that between-species transfer of the
88 plasmid from a proficient host, *P. fluorescens*, to an unstable host, *P. putida*, allowed the plasmid to
89 persist in *P. putida* both with and without mercury selection. This finding suggests that the dynamics
90 of a plasmid in a bacterial community is likely to depend on the proficiency of the plasmid host species
91 to stably maintain the plasmid. This leads to the prediction that, at the community-level, plasmid
92 abundance will be higher in communities where it is carried by a proficient original plasmid host, since
93 this species will both be able to maintain the plasmid in its own population, and then disseminate the
94 plasmid to other species in the community.

95 To test this prediction, we tracked the dynamics of pQBR103 in a three-species community of
96 *P. fluorescens*, *P. stutzeri* and *P. putida* with and without mercury selection. We varied which of the
97 species carried the plasmid at the start of the experiment. We hypothesised that the community-level
98 plasmid abundance would vary according to the proficiency of the original plasmid host species to act
99 as hosts to pQBR103, which varies hierarchically – *P. fluorescens* > *P. stutzeri* > *P. putida* (Kottara *et*
100 *al*. 2018). Replicate communities were propagated in effectively sterile potting soil (artificial soil
101 microcosms), which provide spatial structure and a low resource environment that more closely
102 resemble the natural physical and chemical conditions in soil and promote the stable co-existence of
103 multiple bacterial species (Gómez and Buckling 2011; Heuer and Smalla 2012; Hall *et al*. 2015; Hall
104 *et al*. 2016).
105

106 **MATERIALS AND METHODS**

107 **Bacterial strains and plasmid**

108 Three *Pseudomonas* species – *P. fluorescens* SBW25 (Rainey, Bailey and Thompson 1994), *P. stutzeri*
109 JM300 (DSM 10701) (Busquets *et al*. 2012) and *P. putida* KT2440 (Bagdasarian *et al*. 1981) – were
110 utilised in this study. *Pseudomonas* species were labelled by directed insertion of either streptomycin
111 (Sm^R) or gentamicin resistance (Gm^R) marker using the mini-Tn*7* transposon system (Lambertsen,
112 Sternberg and Molin 2004). The plasmid used in this study, pQBR103 is a large conjugative plasmid
113 (425 kb) that confers mercury resistance via a *mer* operon encoded on a Tn*5042* transposon (Lilley *et*
114 *al*. 1996; Tett *et al*. 2007). pQBR103 plasmid is part of a group of 136 plasmids that were isolated from
115 the bacterial community inhabiting the sugar beet rhizosphere and phyllosphere during a long-term field
116 experiment (Lilley *et al*. 1996). pQBR103 was exogenously acquired by conjugation into labelled strain
117 of *P. fluorescens* that was introduced onto the naturally occurring bacterial community colonising the
118 sugar beet rhizosphere with the primary plasmid-host remaining unknown (Lilley *et al*. 1996). To obtain
119 the initial plasmid-bearing clones of each host species to start the selection experiment, pQBR103
120 plasmid was conjugated into *P. stutzeri* Gm^R, *P. putida* Sm^R and *P. fluorescens* Sm^R*lacZ* from the
121 plasmid-bearing *P. fluorescens* SBW25 Sm^R or Gm^R stocks. Plasmid conjugation was performed by
122 mixing 1:1 each of the plasmid-free with the plasmid-bearing strains, incubating for 48 h and spreading
123 on King's B growth (KB) agar plates containing 5 μg mL^{-1} gentamicin or 50 μg mL^{-1} streptomycin (50
124 μg mL^{-1} X-Gal) and 20 μM of mercury(II) chloride to select for transconjugant colonies (Simonsen *et*
125 *al*. 1990). The conjugation assays were conducted in 6 mL KB medium in 30 mL universal vials
126 ('microcosms') at 28°C in shaking conditions (180 rpm).

127

128 **Selection experiment**

129 To account for the high segregation rate of the plasmid in *P. putida* KT2440 (Kottara *et al*. 2018) and
130 to ensure high starting frequencies of plasmid carriage across all the tested bacterial strains, single
131 colonies of each plasmid-bearing species were reconditioned overnight and then transferred in fresh
132 media containing mercury. Specifically, individual colonies (n=12) of each plasmid-bearing

133 *Pseudomonas* species were picked into separate 6 mL KB microcosms and incubated overnight at 28°C
134 with shaking 180 rpm after which time 1% of each population was transferred to grow for 24 h in fresh
135 KB microcosms containing 50 μM of mercury(II) chloride at same temperature and shaking conditions;
136 this concentration of mercury was used to select for the pQBR103 plasmid based on previous findings
137 (Kottara *et al*. 2018). Similarly, 24 colonies of each plasmid-free *Pseudomonas* species were each
138 grown overnight in KB 6 mL microcosms and transferred to grow for 24 h in fresh KB microcosms at
139 same temperature and shaking conditions.
140
141 *Bacterial communities*
142 We used artificial soil microcosms to evolve three different bacterial communities differing by which
143 species carried the plasmid at the beginning of the experiment (original plasmid host). To prepare the
144 artificial soil microcosms, we added 10 g of John Innes No. 2 compost soil in 30 mL universal vials
145 which we autoclaved twice. By autoclaving the compost soil two times, we established an effectively
146 sterile micro-environment with the physical and chemical properties of soil which did not contain other
147 culturable bacteria than our inoculum (Gómez and Buckling 2010; Hall *et al*. 2015; Hall *et al*. 2016).
148 Three different bacterial communities were then constructed: *P. fluorescens* (carrying pQBR103) with
149 *P. stutzeri* and *P. putida*; *P. fluorescens* with *P. stutzeri* (pQBR103) and *P. putida*; *P. fluorescens* with
150 *P. stutzeri* and *P. putida* (pQBR103). Six replicates of each community were grown either without
151 mercury or with mercury (16 μg g^{-1} Hg(II)); this concentration of mercury was used to select for the
152 pQBR103 plasmid while could allow the survival of the plasmid-free species based on previous findings
153 (Hall *et al*. 2015). After the addition of Hg(II), each artificial soil microcosm was briefly vortexed to
154 ensure homogeneous distribution of mercury in soil. Each community had a starting ratio of 1:1:1 of
155 each *Pseudomonas* species such that the starting frequency of pQBR103 in the community was
156 approximately 33%. To remove spent media and residual mercury from overnight cultures each
157 inoculum was briefly vortexed, then centrifuged for 1 min at 10,000 rpm and resuspended in 1 mL M9
158 salt solution (Sambrook, Fritsch and Maniatis 1989). 100 μL was then inoculated into artificial soil
159 microcosms, briefly vortexed to ensure spread homogeneity in artificial soil microcosms and incubated
160 at 28°C at 80% humidity (Hall *et al*. 2016).

161

162 *Serial transfers and bacterial counts*

163 Every 4 days, 10 mL of M9 buffer and 20 glass beads were added to each artificial soil microcosm and
164 mixed by vortexing for 1 min, and 100 µL of soil wash was transferred to a fresh artificial soil
165 microcosm as previously described by Hall *et al*. (2016). Bacterial counts for each species were
166 estimated by plating onto selective media: 50 µg mL^{-1} streptomycin + 50 µg mL^{-1} X-Gal KB agar plates
167 and 5 µg mL^{-1} gentamicin KB agar plates, each of which was then replica plated onto mercury KB agar
168 plates (100 µM mercury(II) chloride). The bacterial communities were evolved for 10 transfers (~40
169 days, estimated to be approximately 70 bacterial generations).

170

171 *Plasmid and mercury resistance transposon screening*

172 Twenty-four mercury-resistant colonies of each *Pseudomonas* species were sampled every 2 transfers
173 from the mercury containing plates and tested for the presence of the plasmid and mercury resistance
174 transposon by PCR screening. The PCR used the same sets of primers as previously described [*mer*
175 operon on the Tn*5042* transposon – forward primer: 5'-TGCAAGACACCCCCTATTGGAC-3', reverse
176 primer: 5'-TTCGGCGACCAGCTTGATGAAC-3' and origin of replication of the plasmid (*ori*V) –
177 forward primer: 5'-TGCCTAATCGTGTGTAATGTC-3', reverse primer: 5'-
178 ACTCTGGCCTGCAAGTTTC-3'] (Harrison *et al*. 2015; Kottara *et al*. 2018).

179

180 **Statistics**

181 Statistical analyses were performed using RStudio version 3.2.3 (R Core Team 2013). Shapiro-Wilk
182 test, normal Q-Q plots, histograms and box-plots were used to examine the normality of the data. We
183 found that in most cases the data were not normally distributed, and in such cases used a non-parametric
184 test. Cumulative plasmid abundance in each community over time was estimated as the area under the
185 curve using the function *auc* of the package 'flux' (Jurasinski, Koebsch and Hagemann 2012).
186 Community-level plasmid abundances in the plasmid host treatments were compared by using the
187 Kruskal-Wallis test. To assess the plasmid-dynamics within each species, we compared plasmid

188 frequencies in the plasmid-recipient species population as the area under the curve. The integral
189 estimates of the plasmid frequency in the recipient species were compared between the mercury
190 conditions using the Kruskal-Wallis test. To assess the timing of chromosomal acquisition of the
191 mercury resistance transposon Tn*5042* in *P. putida* that differed between the plasmid host treatments,
192 for each population we recorded the transfer number when we first observed plasmid-free transposon-
193 containing genotypes of *P. putida*. We compared these values between the plasmid host treatments
194 using the Kruskal-Wallis test. The species diversity of plasmid-carriers was calculated as the 1 - D
195 Simpson's Index, 1- $[\sum = (\frac{n}{N})^2]$ where, n = the end-point population density of each plasmid-bearer
196 species in community, and, N = the end-point population density of all plasmid-bearer species. We
197 compared diversities between the plasmid host treatments and mercury conditions by using the Kruskal-
198 Wallis test.
199
200 **RESULTS**
201 **Original plasmid host species identity affects community-level plasmid abundance**
202 The bacterial host species vary in their ability to stably maintain pQBR103 hierarchically as follows:
203 *P. fluorescens* > *P. stutzeri* > *P. putida* (Kottara *et al*. 2018). We hypothesised therefore that the identity
204 of the original plasmid host in a community is likely to affect the dynamics of the plasmid-encoded
205 mercury resistance at the community-level. To test this, we quantified the total plasmid abundance in
206 each community (Figure 1). Mercury selection increased total plasmid abundance (effect of mercury;
207 $\mathcal{X}^2$(1, N=24)=17.28, p=3.226e-05) and total plasmid abundance varied with original plasmid host
208 identity, such that both with (effect of plasmid treatment; $\mathcal{X}^2$(2, N=18)=11.556, p=0.003) and without
209 (effect of plasmid treatment; $\mathcal{X}^2$(2, N=18)=11.474, p=0.003) mercury selection, the total plasmid
210 abundance was higher when the original plasmid host was *P. fluorescens*. Together these data suggest
211 that community-level plasmid dynamics are affected by both the positive selection for plasmid-encoded
212 traits and the identity of the original plasmid host species, being enhanced when plasmids are beneficial
213 and carried by a proficient plasmid host.
214

215 **Species-level plasmid dynamics within communities**

216 To understand how the variation in community-level plasmid abundance was driven by original plasmid
217 host identity, we next examined the species-level plasmid dynamics in each community. As predicted,
218 when a proficient plasmid-host – *P. fluorescens* – was the original plasmid host it maintained the
219 plasmid at high frequency within its population both with and without mercury (Figure 2). We detected
220 plasmid dissemination from *P. fluorescens* to the other species at higher frequencies under mercury
221 selection (effect of mercury; $\mathcal{X}^2$(1, N=24)=4.653, p=0.030). This occurred to *P. putida* in all replicates
222 and to *P. stutzeri* in 2/6 replicates with mercury selection and also to *P. stutzeri* at low levels in some
223 of the communities without mercury selection. When *P. stutzeri* was the original plasmid host, it also
224 maintained the plasmid within its own population both with and without mercury, and disseminated
225 plasmids to the other species at a higher rate with mercury (effect of mercury; $\mathcal{X}^2$(1, N=24)=11.644,
226 p=0.0006) (Figure 3). Variation in total plasmid abundance between replicate communities in this
227 treatment appear to have been caused by whether or not *P. fluorescens* acquired the plasmid before it
228 was outcompeted by mercury resistant competitors: where transmission to *P. fluorescens* occurred, total
229 plasmid abundances were higher (Figure 3). Where *P. putida* was the original plasmid host, it did not
230 maintain the plasmid within its own population: without mercury, the plasmid was simply lost, whereas,
231 with mercury, plasmid-bearers were replaced by mutants that had inserted the Tn*5042* encoding the *mer*
232 operon into their chromosome (Figure 4). Chromosomal insertions of the Tn*5042* in *P. putida* were
233 observed in the other plasmid host treatments, but arose much later in these communities where *P.*
234 *putida* had to acquire the plasmid horizontally from either *P. fluorescens* or *P. stuzeri* (effect of
235 treatment; $\mathcal{X}^2$(2, N=18)=10.947, p= 0.004). Although *P. putida* eventually lost the plasmid from its
236 own population, prior to this loss it successfully disseminated the plasmid to *P. fluorescens* in 6/6
237 replicates and to *P. stutzeri* in 3/6 replicates with mercury selection (Figure 4).

238

239 **Diversity of plasmid-carriers in communities**

240 Finally, we tested how the original plasmid host identity affected the diversity of plasmid-carriers at the
241 end of the experiment. The diversity of plasmid-carriers was affected by both the original plasmid host

242 species identity (effect of plasmid treatment; $\mathcal{X}^2$(2, N=36)=12.819, p=0.001) and mercury selection
243 (effect of mercury; $\mathcal{X}^2$(1, N=36)=6.082, p=0.013) (Figure 5). Without mercury selection the diversity
244 of plasmid-carriers was highest when *P. stutzeri* was the original plasmid host. Whereas, with mercury
245 selection, the diversity of plasmid-carriers was higher in communities where *P. fluorescens* or *P.*
246 *stutzeri* were the original plasmid hosts compared to communities where *P. putida* was the original
247 plasmid host. Consistent with our data on community-level plasmid abundance, these data show that
248 the diversity of plasmid-carriers is likely to be higher when plasmids are beneficial and are introduced
249 to the community by proficient plasmid hosts.
250

251 **DISCUSSION**

252 In natural microbial communities, broad host range plasmids are frequently transmitted to diverse host
253 species thus highlighting the importance of plasmids in HGT and their role in the spread of resistance
254 genes in the environment (Pukall, Tschäpe and Smalla 1996; Klümper *et al*. 2015). In this study, we
255 aimed to understand the extent to which plasmid dynamics in a bacterial community are affected by the
256 original plasmid host species identity within that community. Our findings suggest that plasmid
257 abundance at the community-level was driven by the identity of the original plasmid host species. We
258 observed that pQBR103 reached higher community-level abundance when hosted by a proficient
259 plasmid-host, *P. fluorescens*. Dionisio *et al*. (2002) have previously shown the importance of species
260 identity in shaping the plasmid dynamics in a community. This was further described by Hall *et al*.
261 (2016) where a proficient plasmid-host could act as a source of the plasmid for a non-proficient host
262 species in a two-species soil community. These plasmid dynamics were explained in terms of
263 conjugative plasmids persisting in the community as infectious agents via interspecies transfer (Bahl,
264 Hansen and Sørensen 2007). Here, we extend these results to a more complex three-species community,
265 a different plasmid, and a wider range of plasmid host species and proficiencies.

266 The community-level plasmid abundance also varied according to mercury selection. In
267 common with previous studies (Cairns *et al*. 2018), plasmids were observed at higher frequencies in
268 recipient species in the presence versus absence of positive selection. Detecting HGT events is more

269 likely under positive selection, because, while individual conjugation events may be rare, positively
270 selected horizontally acquired genes will rise to high frequency due to clonal expansion. This has led
271 to a generally accepted, but probably incorrect view, that HGT is accelerated under positive selection
272 (Aminov 2011; Fletcher 2015). By contrast, recent experimental data shows that horizontal
273 transmission plays a more important role in plasmid stability in the absence of positive selection
274 (Stevenson *et al*. 2017), leading to higher rates of gene mobilisation and transfer in these environments
275 (Hall *et al*. 2017b). Mercury selection also drove the invasion of *P. putida* mutants that had lost the
276 plasmid but captured the Tn*5042* carrying the mercury resistance operon to the chromosome, an
277 outcome rarely observed in the other host species. This confirms our previous data that the rate and/or
278 propensity for transposition of traits from the plasmid to the chromosome is variable among
279 *Pseudomonas* species (Kottara *et al*. 2018). We show here that the dynamics of this process are affected
280 by the community context, specifically whether or not *P. putida* was the original plasmid host.
281 Chromosomal capture of mercury resistance transposon in *P. putida* occurred earlier when it began the
282 experiment with the plasmid, reflecting that transposition is random mutational event and thus more
283 likely to occur in larger – plasmid-bearing – populations. Interestingly, however, our data also show
284 that even low proficiency plasmid hosts, which rapidly capture useful traits and jettison the plasmid,
285 can act as a source of plasmids for other species in community by transferring the plasmid to more
286 proficient host species before it is lost.

287 In contrast to the study of Hall *et al*. (2016), which used a highly conjugative plasmid, pQBR57,
288 the plasmid used here, pQBR103, has >1000-fold lower conjugation rate (γ, the rate parameter for
289 conjugative plasmid transfer estimated by the end-point method as developed by Simonsen *et al*. (1990);
290 $Log_{10}(\gamma)$ pQBR103= ~ -13.8, $Log_{10}(\gamma)$ pQBR57= ~ -10.8; Hall *et al*. 2015). While previous studies of
291 pQBR103 have focused on the importance compensatory evolution in its longer-term stability (Harrison
292 *et al*. 2015), here we show an effect of between species conjugation increasing the community-level
293 abundance of the plasmid. The role for interspecific conjugation in pQBR103 stability was most notable
294 in communities where it was initially carried by a non-proficient original plasmid host, *P. putida*. Here,
295 while the plasmid started in ~33% of the population and went extinct in the *P. putida* population, it
296 survived in the community by horizontal transmission, most commonly into *P. fluorescens*. Through

297 interspecific conjugation, pQBR103 increased the diversity of plasmid-carriers in communities,
298 especially under mercury selection. However, this effect depended upon the original plasmid host
299 species identity. Conjugation also depends on the population density, and in this case the higher
300 population density of *P. fluorescens* could have enabled the plasmid transfer from *P. fluorescens*.
301 Surprisingly, although with mercury selection more proficient plasmid host species (*i.e. P. fluorescens*
302 and *P. stutzeri*) allowed higher diversities of plasmid-carriers, without mercury it was in communities
303 where the moderately proficient plasmid host, *P. stutzeri*, was original plasmid host where the highest
304 plasmid-carrier diversity was observed. This effect is likely to have been caused by the more equitable
305 distribution of plasmid carriage in these communities, and specifically by higher rates of plasmid
306 carriage in *P. stutzeri* itself compared to communities where this species had to obtain the plasmid via
307 conjugation.

308 Soil microbial communities are highly diverse, which is thought to play a key role in their
309 function (Torsvik and Øvreås 2002) and species diversity has been suggested to play a role in the
310 dissemination of conjugative plasmids (Dionisio *et al*. 2002). Soil habitats are often characterised as
311 hot spots for HGT (van Elsas and Bailey 2002; Sørensen *et al*. 2005) due to the spatially structured
312 nature of such environments (Bahl, Hansen and Sørensen 2007; Fox *et al*. 2008; Røder *et al*. 2013).
313 Here, we show that the identity of original plasmid host species determines the community-level
314 abundance of conjugative plasmids in soil bacterial communities. Proficient plasmid hosts better
315 maintain plasmids within their own population and transmit these plasmids to other species in the
316 community. This implies that proficient plasmid host species could promote the robustness of
317 communities by spreading potentially adaptive genes to more diverse species, allowing their survival
318 upon environmental deterioration in the future.

319

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AUTHOR CONTRIBUTIONS

AK, JH and MB designed the study; AK performed the experiments and analysed the data; AK and MB drafted the manuscript.

Conflict of interest. The authors declare that there are no conflicts of interest.

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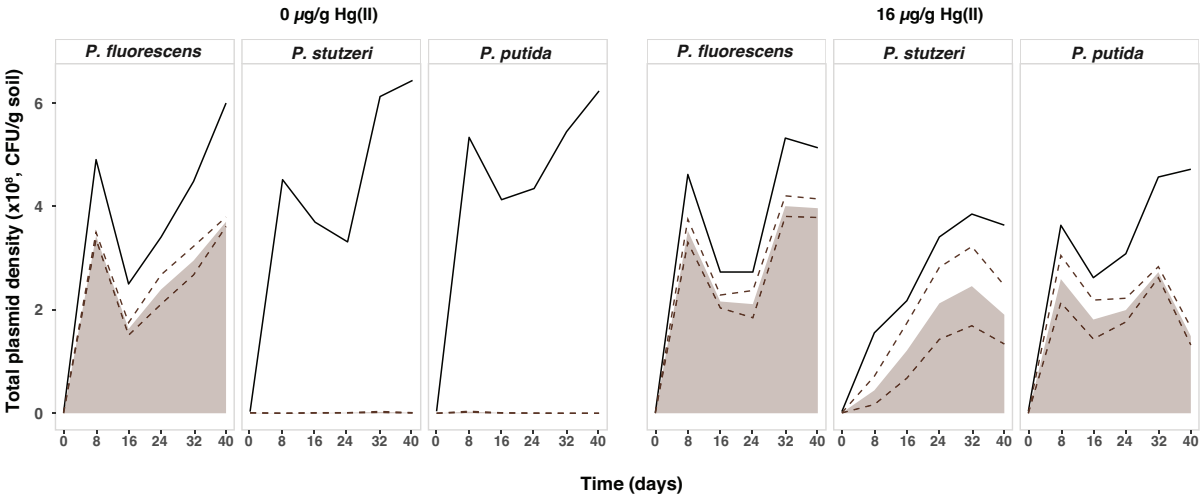


428

429 **Figure 1.** Total plasmid density in the community throughout the selection experiment. Panels
430 data for communities that varied in mercury selection (without mercury, left-hand set; with
431 mercury, right-hand set) their initial original plasmid host (from left to right in each set: *P.*
432 *fluorescens*, *P. stutzeri* or *P. putida*). Brown shaded area shows the mean plasmid abundance
433 in the community ± standard error (dotted line) from six replicates. Solid lines show the mean
434 total community bacterial density from six replicates.
435

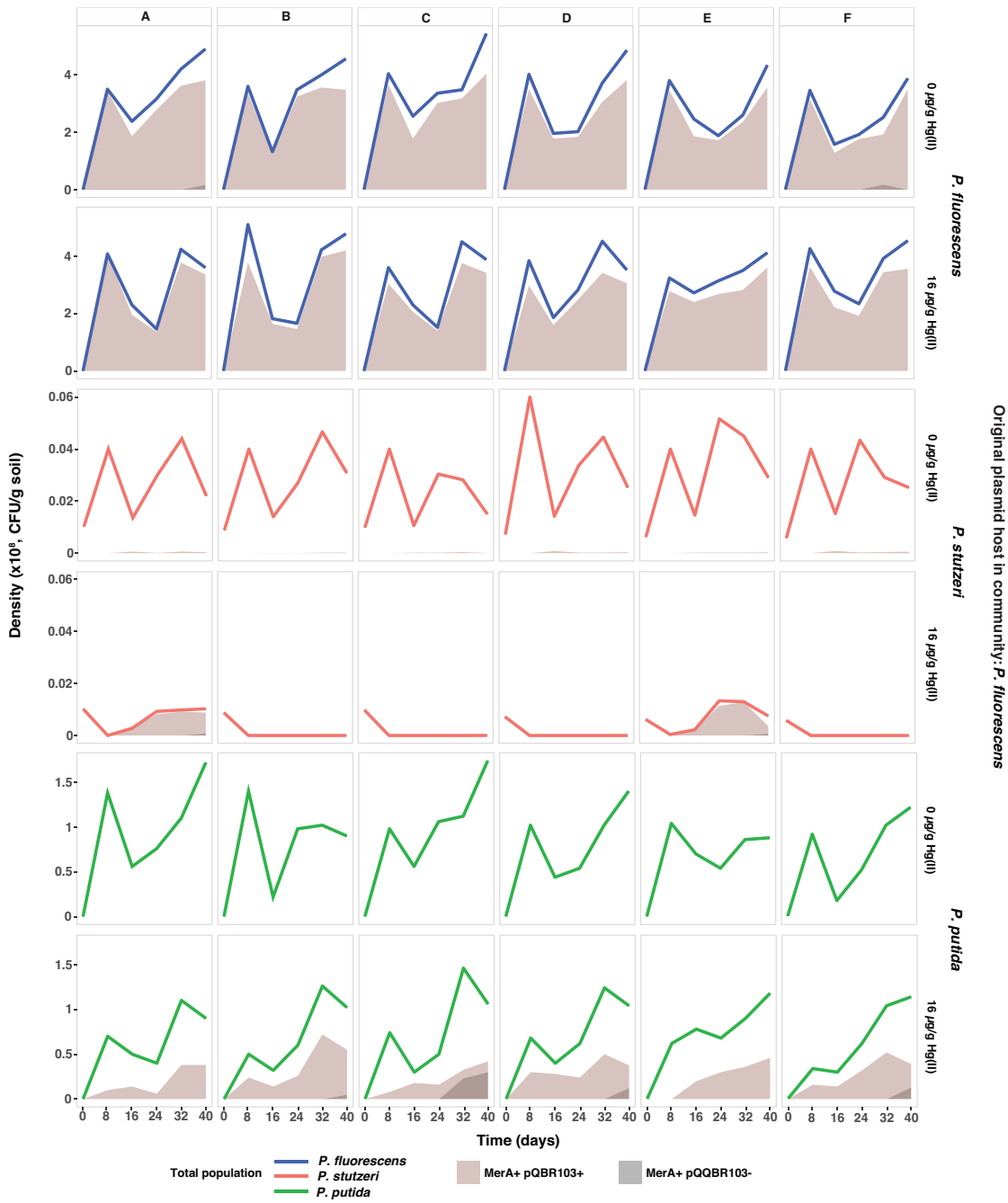
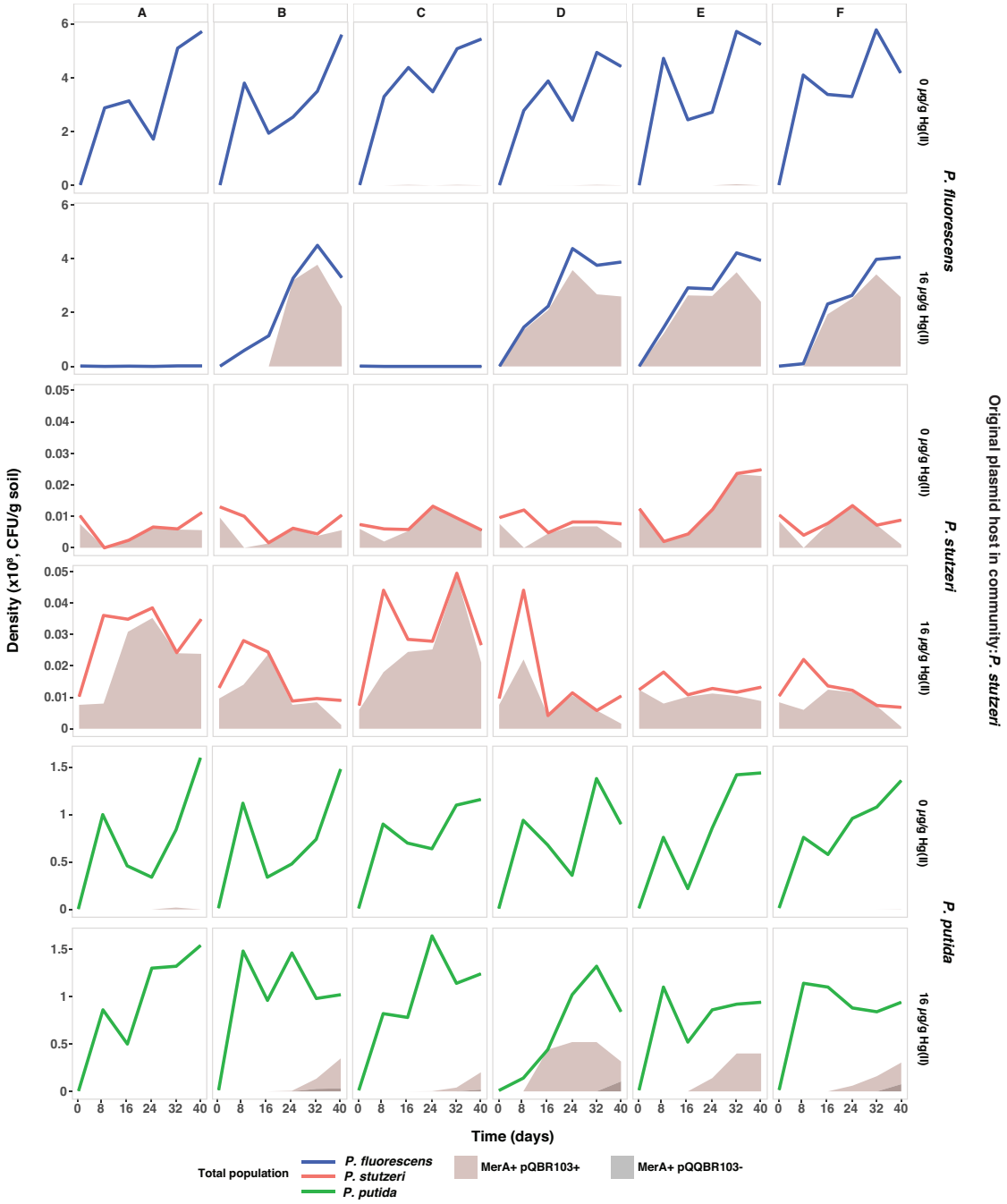


Figure 2. Population density and mobile genetic element dynamics in communities where ***P. fluorescens*** was the original plasmid host. A-F clonal populations evolving with or without mercury. Lines show the population densities of *P. fluorescens* (blue); *P. stutzeri* (red); *P. putida* (green). Brown areas show the density of plasmid carriers; Grey areas show the density of cells that have retained the Tn5042 but lost the plasmid.



443

444 **Figure 3.** Population density and mobile genetic element dynamics in communities where ***P.***
445 ***stutzeri*** was the original plasmid host. A-F clonal populations evolving with or without
446 mercury. Lines show the population densities of *P. fluorescens* (blue); *P. stutzeri* (red); *P.*
447 *putida* (green). Brown areas show the density of plasmid carriers; Grey areas show the density
448 of cells that have retained the Tn5042 but lost the plasmid.

449

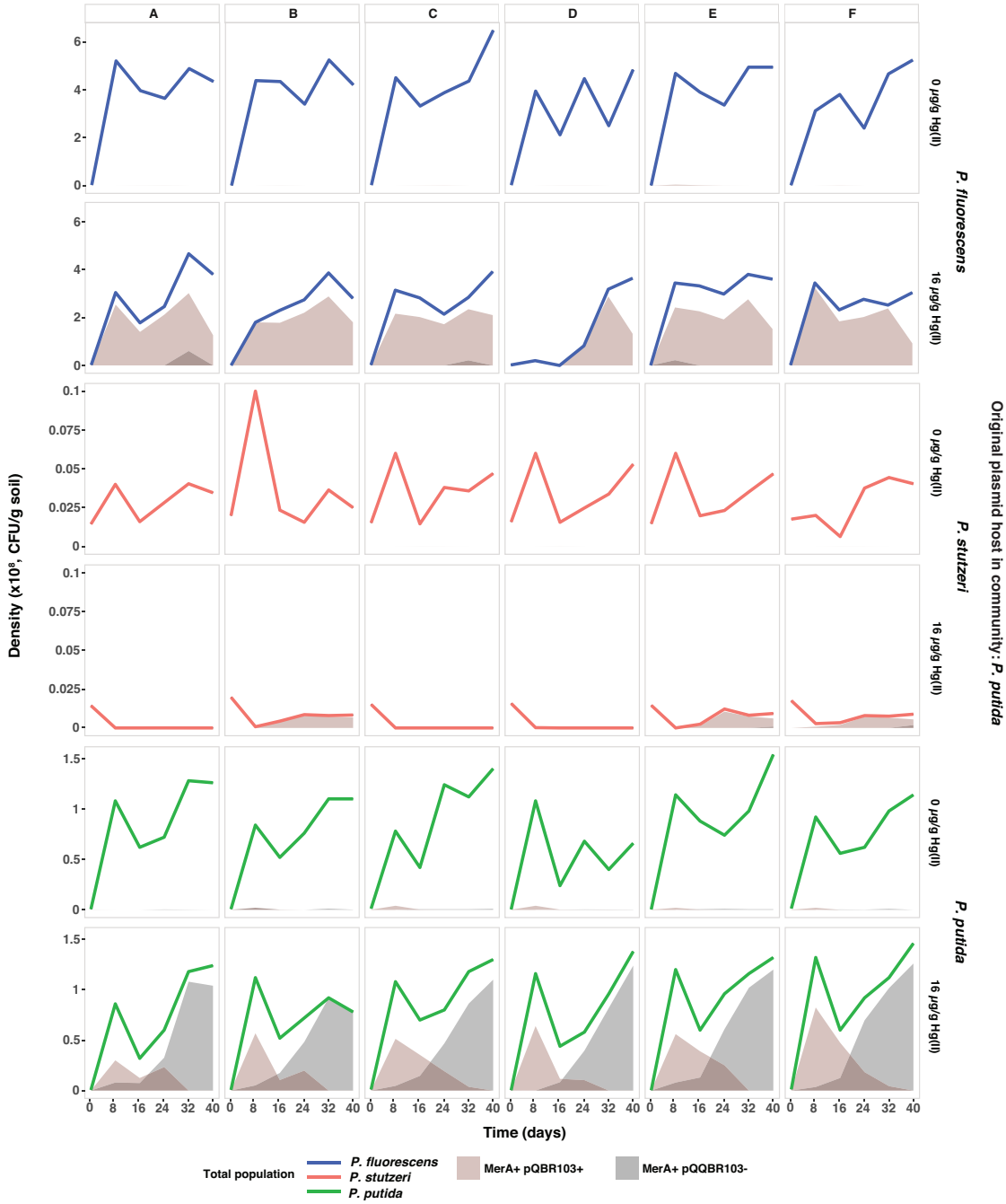
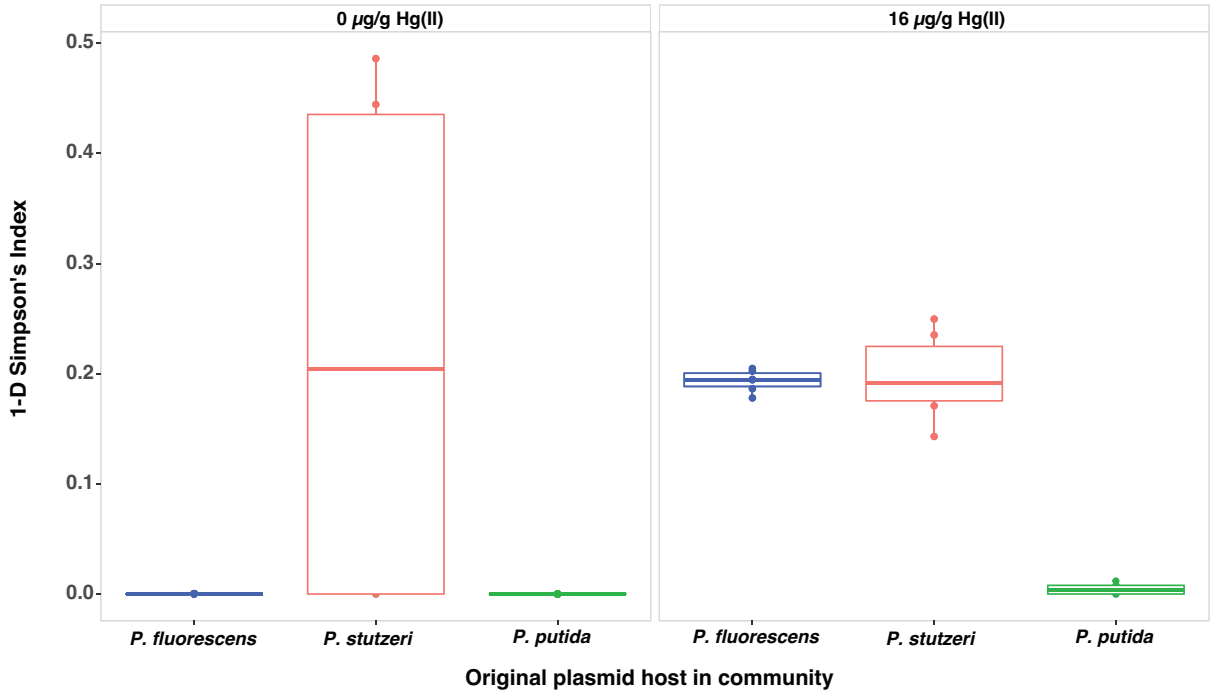


Figure 4. Population density and mobile genetic element dynamics in communities where ***P. putida*** was the original plasmid host. A-F clonal populations evolving with or without mercury. Lines show the population densities of *P. fluorescens* (blue); *P. stutzeri* (red); *P. putida* (green). Brown areas show the density of plasmid carriers; Grey areas show the density of cells that have retained the Tn5042 but lost the plasmid.



457

458 **Figure 5.** Diversity of plasmid-carriers at the end of the experiment. Species diversity was
459 calculated as the 1-D Simpson's Index by using the end-point population densities of the
460 plasmid-carriers in each species in each original plasmid host community.

461