



HAL
open science

Horizontal gene transfer among host-associated microbes

Jorge Moura de Sousa, Marta Lourenço, Isabel Gordo

► **To cite this version:**

Jorge Moura de Sousa, Marta Lourenço, Isabel Gordo. Horizontal gene transfer among host-associated microbes. *Cell Host & Microbe*, 2023, 31 (4), pp.513 - 527. 10.1016/j.chom.2023.03.017 . hal-04604566

HAL Id: hal-04604566

<https://hal.science/hal-04604566>

Submitted on 7 Jun 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 Horizontal gene transfer among host-associated 2 microbes

3

4 Jorge Moura de Sousa^{1,4}, Marta Lourenço^{2,4} and Isabel Gordo^{3,4,*}

5

6 1 Institut Pasteur, Université Paris Cité, CNRS, UMR3525, Microbial Evolutionary
7 Genomics, Paris 75015, Paris, France

8 2 Institut Pasteur, Université Paris Cité, Biodiversity and Epidemiology of Bacterial
9 Pathogens, F-75015, Paris, France

10 3 Instituto Gulbenkian de Ciência, Rua da Quinta Grande,6, Oeiras, Portugal

11 4 These authors contributed equally

12 *Correspondence: igordo@igc.gulbenkian.pt

13

14 In brief

15 Bacterial evolution is highly influenced by events of horizontal gene transfer. We review recent
16 studies on mechanisms of transfer and the complex interactions that these may entail in
17 driving eco-evolutionary dynamics of microbiomes. Integrated approaches are needed to
18 overcome the challenge of quantifying rates and effects of transfer within hosts.

19

20 Abstract

21 Horizontal gene transfer is an important evolutionary force, facilitating bacterial diversity. It is
22 thought to be pervasive in host-associated microbiomes, where bacterial densities are high
23 and mobile elements are frequent. These genetic exchanges are also key for the rapid
24 dissemination of antibiotic resistance. Here, we review recent studies that have greatly
25 extended our knowledge of the mechanisms underlying horizontal gene transfer, the
26 ecological complexities of a network of interactions involving bacteria and their mobile
27 elements, and the effect of host physiology on the rates of genetic exchanges. Furthermore,
28 we discuss other, fundamental challenges in detecting and quantifying genetic exchanges *in vivo*,
29 and how studies have contributed to start overcoming these challenges. We highlight the
30 importance of integrating novel computational approaches and theoretical models with
31 experimental methods where multiple strains and transfer elements are studied, both *in vivo*
32 and in controlled conditions that mimic the intricacies of host associated environments.

33 Introduction

34

35 All animals and plants on the planet are colonized by complex microbial communities, also
36 called microbiomes. The characterization of host-associated microbiomes, and in particular
37 those that are human-associated, has seen an increase in research focus. This is partially due
38 to the realization that the expression and evolution of host traits can be affected by the
39 composition and stability of their microbiomes^{1,2}. Thus, human, animal and plant health can
40 be affected both negatively and positively by the disruption of their associated microbial
41 communities.

42

43 Microbes colonize many different niches within a host. In the human body, for instance,
44 microbes are found in many areas, including the oral cavity, the gastrointestinal tract, the skin
45 and even the lungs. Each of these niches comprises a distinct environment, that may be
46 suitable for certain microbial types but not others. Furthermore, microbiomes are not static.
47 Besides the invasion or extinction of certain microbial species, both the composition and
48 functionality of microbiomes can change as bacteria adapt. Both bacterial evolution and
49 migration to new host-associated niches can change their relationship with the host, even
50 turning commensals into pathogens³. Evolutionary change in microorganisms can occur either
51 by random mutations or by horizontal gene transfer (HGT), a major process that shapes within
52 and between species diversity in bacterial populations. Unlike mutation, which typically
53 involves small genetic changes in the bacterial genome that are vertically inherited, HGT
54 transfers genetic material between different bacterial clones. This is predominantly driven by
55 numerous mobile elements that are ubiquitous in the bacterial genomes⁴.

56

57 Most of our knowledge about HGT comes from *in silico* (i.e., comparative genomics) and *in*
58 *vitro* studies, for which the latter typically represent experimental simplifications of more
59 complex biological systems. While there is evidence that point mutations, small deletions or
60 transposable element insertions can occur at an appreciable rate *in vivo*⁵, quantifying, or even
61 detecting HGT in host-associated microbiomes is hindered by several challenges. First, the
62 interactions that occur within and between species in microbiomes entangle ecology, evolution
63 and epidemiology. These interactions complicate the identification of the relevant evolutionary
64 parameters that underly each of the individual processes. Second, the intense activity of a
65 network of selfish mobile genetic elements (MGEs), affects not only bacterial survival, but also
66 hinder and promote genetic exchanges between cells. Finally, the host environment where
67 microbiomes evolve pose a challenge on its own. Its complex physiology, biotic and abiotic

68 factors, including antibiotics, result in environmental conditions that are a far-cry from the
69 simplifications typically studied *in vitro*.

70

71 Recently, multidisciplinary approaches have contributed to understanding the importance and
72 prevalence of HGT *in vivo*. Yet, a large part of the puzzle is still missing. In this review, we will
73 discuss the advances, complexities and challenges in both detecting and quantifying HGT in
74 microbial communities from host-associated environments, as well as the host factors that
75 may modulate the evolutionary dynamics. We highlight potential integrative strategies that will
76 play an important role in uncovering the impact of HGT within hosts and, consequently, in
77 global health.

78

79 The elements and mechanisms that drive horizontal 80 gene transfer

81

82 The main mechanisms of HGT are transformation, conjugation, transduction and lysogeny
83 (Figure 1). Transformation involves the uptake of external DNA by a cell, and its genomic
84 integration by homologous recombination. Transformation has multiple effects. Although the
85 probability of integrating a novel sequence through homologous recombination declines
86 exponentially with the level of sequence divergence⁶, transformation can contribute to genetic
87 exchanges between bacteria⁷. On the other hand, because transformed DNA originates from
88 dead bacteria, it has been argued that transformation events can be deleterious for the cell⁸.
89 It is possible that one of the main roles of transformation is to act as a mechanism of genomic
90 resilience. Uptaken sequences can be used as templates to repair deleterious mutations that
91 occur during the replication of bacterial chromosomes, or as a way to inactivate costly MGEs⁹.
92 It is difficult to accurately track transformation events in bacterial chromosomes, and its role is
93 still poorly studied *in vivo*. This is partially due to the technical limitations in actually detecting
94 such genetic exchanges, due to the strong homology required for recombination with uptaken
95 DNA. Nevertheless, transformation occurs at high rates in some bacterial species like
96 *Neisseria*¹⁰ and *Hemophilus*¹¹, and many species have competence genes¹². Hence,
97 transformation might have an important evolutionary role for bacteria in particular
98 environmental conditions.

99

100 Transformation is limited by the short time that DNA remains intact outside the cell. Some
101 bacteria can produce different types of membrane vesicles (MV) or outer membrane vesicles
102 (OMV), and these nanostructures can package proteins, metabolites, and toxins present in
103 the bacterial cell¹³. Both DNA and RNA can also be packaged within these extracellular
104 vesicles, and are thus much better protected from environmental degradation. MGEs can also
105 be packaged within MVs and OMVs, as observed for *K. pneumoniae*¹⁴ and *Ruminococcus*¹⁵.
106 This suggests that such structures might play an important, yet still understudied role during
107 HGT *in vivo*.

108

109 Other HGT mechanisms rely on the different types of MGEs¹⁶ (see Figure 1) that populate
110 bacterial cells¹⁷, and that are relatively easier to detect. Conjugation involves the movement
111 of plasmids or integrative conjugative elements (ICEs, that contrary to plasmids integrate
112 bacterial genomes) through close contacts between cells of the same or of different species.

113 Transduction and lysogeny mainly involve bacteriophages (phages), viruses that infect
114 bacteria. Contrary to conjugation, phage-mediated HGT allows genetic exchange to occur at
115 larger physical distances between bacteria, as the DNA travels within viral capsids that protect
116 it. Despite the role of MGEs in driving the expansion of gene repertoires in bacteria, the
117 relationship with their bacterial hosts is not always peaceful. Both plasmids and phages have
118 their own lifecycles, and because they are replicating entities with evolutionary interests, they
119 can be bacterial antagonists.

120

121 The burden of MGEs for the fitness of bacteria is more evident in the case of phages, as in
122 order to replicate, they often kill their host bacteria. The two mechanisms of HGT associated
123 with phages are a consequence of their lifecycle. Phages can be virulent or temperate, but
124 only the latter are capable of lysogeny, as they can integrate the bacterial chromosome. When
125 integrated as prophages, they are transmitted vertically upon host replication. The genes they
126 encode can be used by their bacterial hosts (lysogenic conversion). For instance, cholera
127 outbreaks can be caused by toxins encoded in prophages¹⁸, and many other virulence factors
128 are encoded by prophages (e.g.) in marine *Vibrio* communities¹⁹. For certain taxonomic
129 groups, it has been estimated that ca. 50% of all bacterial genomes have at least one
130 prophage²⁰ and this number rises to almost 80% in bacteria from the human gastrointestinal
131 tract²¹. When integrated, prophages may eventually enter a lytic cycle that results in the death
132 of their hosts. During excision and replication, some prophages can include segments of the
133 bacterial chromosome within their viral particles, resulting in generalized, specialized or lateral
134 transduction. In generalized transduction, bacterial DNA from anywhere in the chromosome
135 can fully package viral particles, while specialized transduction packages genes that are in the
136 neighbourhood of integrated prophages, in addition to the prophage itself²². Lateral
137 transduction relies in the replication of phages *in situ*, whilst still integrated, and can result in
138 the transfer of large segments of bacterial DNA that neighbour the prophage²³. Specialized
139 and lateral transduction are thought to be exclusive of temperate phages, while generalized
140 transduction can occur with either temperate or virulent phages. Transduction is thought to
141 arise due to mistakes in the packaging of the viral DNA, although it has been proposed that
142 temperate phages can benefit from such mistakes²⁴. Since the rates of generalized
143 transduction vary between some phages²⁵, transduction may be a trait under selection.

144

145 The induction of prophages in a small fraction of a lysogenic population can lead to the
146 predation of ecological competitors of the lysogens, contributing to the successful invasion of
147 the former²⁶. For many host-associated species, including the pathogenic *Pseudomonas*
148 *aeruginosa*²⁷ or *Klebsiella pneumoniae*²⁸, this may be an important competitive strategy. When
149 the induced phages are also capable of transduction, they can encapsidate DNA from the

150 targeted bacteria and transfer it back to the lysogen population – “autotransduction”. This
151 process allowed a population of antibiotic sensitive lysogens of *Staphylococcus aureus* to
152 invade and acquire antibiotic resistance (AR) genes from a resident (phage sensitive)
153 population both *in vitro* and *in vivo*, within *Galleria mellonella* larvae²⁹. Infection by phages
154 depends on specific cellular receptors (e.g., capsules or lipopolysaccharides), as well as on
155 cellular defenses³⁰. Hence, most phages are thought to have a limited host range (e.g.,³¹),
156 which also limits the reach of phage-mediated HGT.

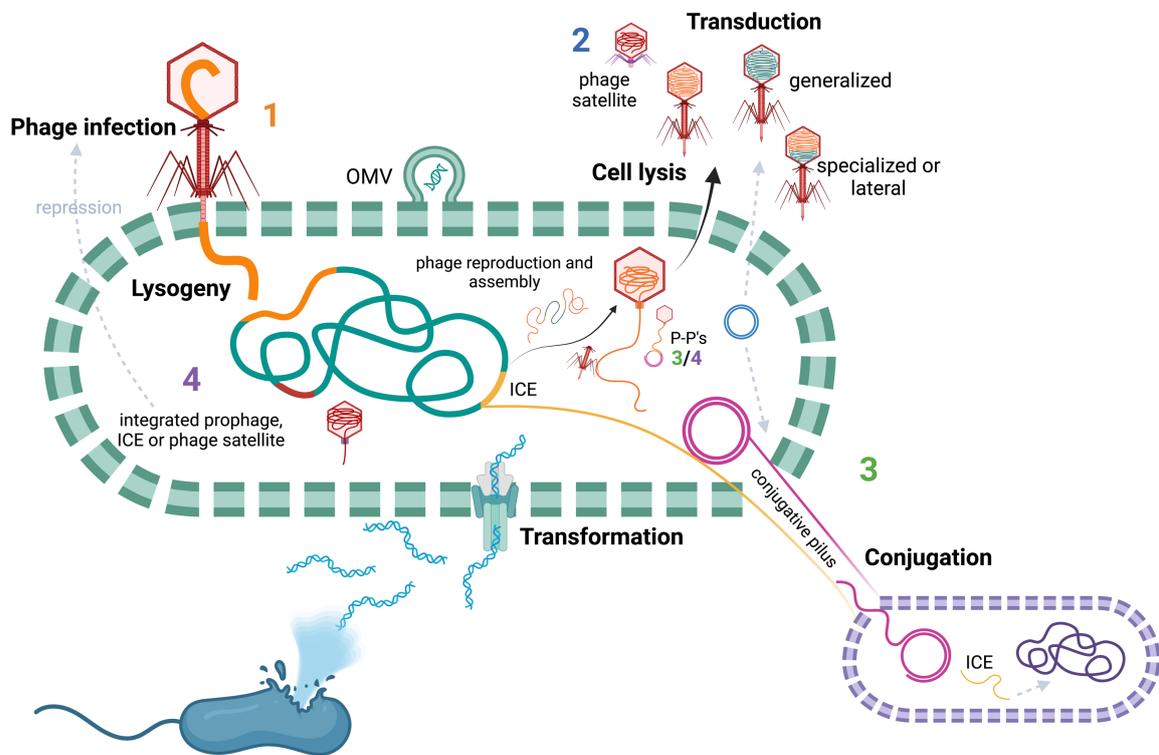
157

158 Conjugative plasmids are extrachromosomal sequences that control their own mobilization.
159 Plasmids encode mating pair formation (MPF) genes responsible for pilus biogenesis and
160 mating junctions, allowing the transfer of the plasmid from the bacterial donor to a recipient
161 one. ICEs also encode these genes, but are integrated in bacterial genomes and can also be
162 stably propagated by vertical transmission. Like prophages, both ICEs and plasmids can
163 encode genes not directly related to their reproduction or transmission, and that increase the
164 fitness of their bacterial host. Conjugation requires close cell-to-cell contact, and it can occur
165 at very high rates, across very distant species (since conjugation does not require specific cell
166 receptors)³², and can transfer large segments of bacterial DNA³³. However, plasmids can
167 impose an important fitness cost to bacteria³⁴, due to the replication of extra DNA, genetic
168 incompatibilities between the genes expressed by the host and those from the plasmid or
169 plasmid-encoded addiction systems that guarantee its stability upon cell division. This means
170 that, like phages, plasmids have a complicated relationship of dependency, parasitism and in
171 some cases, mutual benefits with their hosts.

172

173 Phages and plasmids can themselves be exploited by other mobile elements. Phages are
174 parasitized by phage satellites, small mobile elements that integrate bacterial genomes.
175 Phage satellites differ from phages because they lack the genes to form viral particles. In order
176 to mobilize, phage satellites steal viral particles from “helper” phages that co-infect the same
177 bacterial host³⁵. Phage satellites were recently shown to be very abundant and diverse³⁶. Four
178 main families of phage satellites have been identified, that besides having a common general
179 strategy for mobilization (phage exploitation), are genetically distinct, found in different
180 bacterial hosts, and rely on different specific hijacking mechanisms. P4-like phage satellites
181 are found in many Enterobacteria, and particularly in *E. coli* genomes, which can encode up
182 to three of these satellites^{36,37}. Phage-inducible chromosomal islands (PICI)³⁸ and capsid-
183 forming PICI (cf-PICI, phage satellites that encode their own capsid but still hijack the tails
184 from helper phages)³⁹ are widespread both in Proteobacteria and Firmicutes. On the other
185 hand phage-inducible chromosomal island-like elements (PLE) are exclusively found in *Vibrio*
186 *cholerae* genomes⁴⁰. Phage satellites have an important role in bacteria and can limit, or even

187 completely abolish, phage amplification (see below). They can also play a direct role in
 188 expanding the bacterial gene repertoires, since they can encode virulence and AR genes⁴¹,
 189 or anti-phage defense systems^{42,43}.
 190
 191



	Main-type	Self-mobilizable	Main involvement in HGT processes	Localization in bacteria
1 - Bacteriophages	Virulent, temperate	Yes	Lysogeny (temperate), Generalized transduction (virulent and temperate), Specialized and lateral transduction (temperate)	Lytic infection/cytoplasm (virulent, temperate), chromosome (temperate)
2 - Phage satellites	P4-like, PLE, PICl, cf-PICl	No	Lysogeny, anti-MGE defenses	Chromosome
3 - Plasmids	pConj, pMOB, pOriT	Yes (pConj), No (pMOB, pOriT)	Conjugation, anti-MGE defenses	Extrachromosomal/episomal
4 - Integrative elements	ICE, IME	Yes (ICE), No (IME)	Conjugation, anti-MGE defenses	Chromosome

192
 193 **Figure 1. Mobile genetic elements and the main mechanisms of horizontal gene transfer.** Schematic
 194 representation of the different MGEs and how they drive HGT. Phages can be temperate (i.e., lysogenize bacteria
 195 and become integrated as prophages) or virulent (only perform lytic infections). They infect bacteria and can
 196 perform generalized, specialized or lateral transduction. Phages can be hijacked by phage satellites, that steal viral
 197 capsids from fully functional phages. Satellites can belong to different families (the most well-known are P4-like,
 198 PICl, cf-PICl and PLE). Conjugation occurs when bacteria transfer DNA by direct contact via a conjugative pilus.
 199 It involves the transfer of extrachromosomal sequences typically known as plasmids. Some plasmids are
 200 autonomous transferred (conjugative plasmids, or pConj) whilst others (mobilizable plasmids – pMob – and oriT
 201 plasmids – pOriT) need to co-incide with other conjugative plasmids in order to mobilize. Integrative conjugative
 202 elements (ICEs) are mobile elements that transfer by conjugation and integrate the bacterial chromosome.
 203 Integrative mobilizable elements (IMEs) use the conjugation machinery of ICEs and/or conjugative plasmids to
 204 mobilize. Phage-plasmids (P-Ps) are temperate phages capable of an episomal lifestyle, like plasmids, but that are
 205 transferred within viral particles. Finally, HGT can also occur due to transformation of external DNA, and
 206 extracellular vesicles (like membrane vesicles – MV – or outer membrane vesicles – OMV), nanostructures that
 207 can export both transformable DNA, or even other MGEs.

208

209

210 Plasmids also have their own parasites. The conjugation apparatus of conjugative plasmids
211 and ICEs can be exploited by mobilizable plasmids (pMob)⁴⁴, that encode only an oriT (a small
212 DNA sequence that signals the initiation of the transfer of the plasmid sequence) and a
213 relaxase (responsible for the DNA nick required for initiating the transfer process). pMob are
214 transferred by the conjugative pilus formed by other co-occurring plasmid(s). Both pMob and
215 conjugative plasmids can also be exploited by plasmids that encode only an oriT (pOriT).
216 These were recently shown to be very abundant, corresponding to 49% and 18% of the all the
217 plasmids in *Staphylococcus aureus* and *E. coli*, respectively⁴⁵. Integrative mobilizable
218 elements (IMEs) also use the machinery from other conjugative elements to transfer across
219 cells. IMEs encode only their excision and integration functions, but can have other genes that
220 can increase the fitness of their hosts, including antibiotic resistance genes, toxins, and
221 bacterial transporters and regulators⁴⁶.

222

223 A fraction of non-conjugative plasmids were also recently reclassified as phage-plasmids (P-
224 Ps)⁴⁷, which are an example of the fluidity between MGEs, that defies strict classifications¹⁷.
225 P-Ps are considered temperate phages capable of an episomal, plasmid-like lifestyle. They
226 have the typical replication and segregation functions associated with plasmids, but they are
227 transferred within viral particles that they encode themselves. P-Ps can thus produce virions,
228 lyse cells and infect other hosts that are farther away, unlike the close proximity that is required
229 for conjugation. This flexibility makes them potentially powerful drivers of HGT. For instance,
230 a P1-like P-P in *E. coli* was shown to transfer an extended spectrum beta-lactamase through
231 lysogenic conversion⁴⁸, highlighting the potential of P-Ps in affecting global health.

232

233 The distribution of MGEs in bacterial genomes is shaped by many cellular defense systems
234 that protect the bacterial populations from their invasion or establishment. Knowledge on the
235 abundance, diversity and function of these anti-MGE defense systems has increased
236 significantly (see⁴⁹ for a recent review), and new computational methods systematically
237 identify many known defense systems in bacterial genomes⁵⁰. This will contribute to
238 characterize the distribution of anti-MGE defenses in host associated microbiomes. For some
239 defense systems, their mechanistic understanding is still lacking. Other anti-MGE systems are
240 part of the cell machinery, and are better described. For instance, RecBCD, an exonuclease
241 that repairs double-strand breaks by homologous recombination, can also protect against
242 invasion of some phages⁵¹. Another example are Restriction-modification (R-M) systems, that
243 likely evolved as broad bacterial immune systems. By imprinting bacterial genomes with
244 epigenetic marks, R-M systems defend the bacteria against foreign DNA (e.g., MGEs that lack

245 these epigenetic marks)⁵². Importantly, MGEs can counter these defenses by acquiring them
246 from bacterial genomes (e.g., through homologous recombination). Thus, many MGEs encode
247 anti-MGE defenses that protect the bacteria from other, competitor MGEs^{42,53}. The MGEs can
248 also themselves *be* the defense systems, as is the case for some phage satellites⁴⁰. In either
249 case, this means that systems that protect bacteria against MGEs can be mobilizable, and
250 consequently, HGT itself can modify the frequency of subsequent HGT events.

251

252 Despite the defense mechanisms of bacteria, MGEs are pervasive in the genomes of many
253 species. For instance, in ca. 1300 strains of *Escherichia* from environmental, human and non-
254 human associated hosts' samples, MGEs account for more than a third of the pangenome
255 (the collection of genes) of this species⁵⁴. Given that many of these MGEs code for the
256 information that controls for how (viral particles or conjugation) and when (induction or pilus
257 formation) they are mobilized, it is important to consider the MGEs' interests and evolution
258 when studying HGT in the bacterial hosts. Furthermore, in a similar way to how competition
259 and cooperation between bacteria contribute to the diversity of bacterial communities,
260 interactions between MGEs can also impact the diversity of host-associated microbiomes.

261

262 Competition and cooperation between mobile 263 genetic elements

264

265 Given the abundance and diversity of MGEs, one could easily think that “the more the merrier”
266 for HGT. However, it is becoming increasingly evident that this is not the case, as often there
267 are strong conflicts not only between bacteria and their MGEs, but also between the MGEs
268 themselves⁴. This has consequences for the rates of HGT in bacteria⁵⁵, either directly, or
269 indirectly, by shaping the distribution of MGEs in bacteria.

270

271 An MGE can prevent or diminish the infection, replication or mobilization of other mobile
272 elements, and thus can influence the latter's stability within populations. The fitness costs of
273 mobile parasites for the MGEs they hijack can change with environmental, genetic and
274 epidemiological circumstances^{38,56}. For instance, in *V. cholerae*, PLEs completely abolish the
275 replication of their helper phage, the virulent phage ICP1, by aborting the infection process
276 and killing the bacterial host, effectively halting the propagation of the phage⁴⁰. For this reason,
277 PLE is considered an anti-phage defense system that plays a key role in the ICP1-*V. cholerae*
278 interactions. Some P4-like satellites and PICIs encode specific anti-phage defense systems
279 such as R-M⁴² or abortive infection⁴³. These satellite-encoded defense systems typically do

280 not target their helper phages, but other infecting phages, promoting the survival of the cell
281 and its prophages. Likewise, many plasmids often encode CRISPR-Cas systems, that target
282 other MGEs, particularly phages⁵³. Further studies on these and other anti-MGE mechanisms
283 will likely lead to new insights on the networks of exclusion between MGEs.

284

285 There is also evidence that some MGEs can cooperate, which can result in a more efficient
286 mobilization of the MGEs and increase the rates of HGT. For instance, PICI in *S. aureus* can
287 direct the transfer of unlinked chromosomal regions that contain sequences similar to the
288 packaging (*pac*) sites of the PICI, suggesting a direct role of phage satellites in transduction
289 of bacterial genes through the hijacking of helper phages⁵⁷. Additionally, in *S. aureus*, small
290 non-mobilizable plasmids can be transferred by generalized transduction of both phages and
291 PICIs, suggesting that some plasmids evolved according to the size constraints of phage
292 capsids⁵⁸. Indeed, evidence is mounting that the evolution of MGEs has been shaped by the
293 presence of other elements. A striking example is the peptide-based communication system
294 encoded by some prophages of *Bacillus subtilis*, allowing different phages to coordinate and
295 control their induction⁵⁹ or lysis-lysogeny decision⁶⁰. Hence, HGT through the generation of
296 new lysogens can also be controlled by communication between phages.

297

298 Given the plethora of MGEs, both those known and those yet to be discovered, many
299 interactions are likely to be unknown. The different scales at which interactions can occur,
300 combined with the different MGEs that can engage in these interactions, likely results in
301 complex events. As an example, the phage satellite SaPI3 is induced not by a phage, but
302 instead by another phage satellite, after which they both hijack the viral particles of the helper
303 phage of the latter⁶¹. Furthermore, the interactions between MGEs can be affected by
304 compensatory evolution, when these elements are costly for the host cell. Compensatory
305 mutations might lead to more stable MGEs in bacterial populations, impacting their distribution
306 in microbial communities. Hence, both bacteria-MGE interactions and MGE-MGE interactions
307 might contribute to the frequency and outcomes of HGT *in vivo*, and consequently to the
308 diversity of microbiomes.

309

310

311 **Factors in host-associated environments that shape**

312 **HGT**

313

314 Host-associated environments are the physical niches in an animal or plant, each with different
315 sub-sections, host cell types and physical properties. Despite the control that MGEs exert
316 over their own mobilization, animal/plant host-specific factors can also act as cues that impact
317 certain modes of transfer. Both biotic and abiotic conditions can influence the rate and
318 efficiency of HGT (**Figure 2**). For instance, the gastrointestinal microbiomes from humans in
319 certain industrialized countries show higher rates of gene transfer⁶². The particular cause, or
320 causes, underlying such differences are yet to be determined, but many factors intrinsic to
321 within-host environments factors have the potential to shape HGT processes.

322

323 The spatial structure of host-associated environments impacts bacteria-bacteria and bacteria-
324 MGE interactions⁶³. Relative to the typically studied *in vitro* homogeneous environments,
325 spatial structure brings physical characteristics that can affect the rates of HGT. In many host-
326 associated environments, like plant roots or the mammalian gastrointestinal tract, microbial
327 communities are densely packed and the close cell proximity might lead to high rates of
328 conjugation. Indeed, in the mammalian intestine, *E. coli* can transfer the plasmid TP114 at
329 estimated rates of 0.1 day^{-1} ⁶⁴ and *Salmonella enterica* conjugates the plasmid p2kan with
330 100% efficiency after 4 days of gut colonization⁶⁵. Importantly, plasmid conjugation rates *in*
331 *vivo*, or even their ability to conjugate at all, do not necessarily correlate with *in vitro*
332 measures⁶⁴. This suggests a strong interplay between the type of plasmid, the donor and
333 recipient bacteria strains and even the genetic background of the animal host⁶⁶. Additionally,
334 the spatial structure *in vivo* is expected to affect phage-driven HGT, since the ecological and
335 evolutionary dynamics of phage in structured environments differ from those in homogeneous
336 contexts⁶⁷. Phages are scarcely found in certain niches of host-associated environments, such
337 as the mucosal areas of the mammalian gut, which can serve as refuges for bacteria^{68,69}. This
338 spatial segregation can lead to source-sink dynamics different from the typical co-evolutionary
339 arms-races between phages and bacteria observed *in vitro*⁶⁹.

340

341 The turn-over of bacterial species within microbiomes, due to adaptation, competition, novel
342 colonizations or abiotic factors can lead to an asymmetry on the phage population infectivity
343 at both spatial and temporal scales⁷⁰. It has been suggested that this turn-over might favour a
344 temperate phage lifecycle over a virulent one⁷¹, with the latter favoured in environments with
345 a steadier supply of naïve hosts (e.g., in marine environments). This might contribute to
346 explain the high prevalence of lysogens in gastrointestinal microbiomes, which can drive high
347 rates of transduction and lysogeny in these communities^{21,72}. Interestingly, several bacteria
348 typically found in host-associated environments encode phage satellites^{36,41}, but the
349 prevalence and exchange of these elements *in vivo* has yet to be elucidated. One might

350 speculate that the intense viral dynamics in the gut environment⁷² also potentiate the active
351 exchange of phage satellites.

352

353 The phylogenetic diversity of host-associated microbiomes can vary across niches. For
354 example, gut microbiomes typically contain a higher diversity than vaginal microbiomes⁷³, and
355 the skin microbiome shows a compartmentalization of different sub-communities in spatially
356 separated pores, each with low diversity⁷⁴. This means that even if (e.g.) the rates of
357 conjugation might be high, the potential for gene diversification through conjugation should
358 vary across different sites. Conjugation in diverse communities is an opportunity for bacteria
359 to acquire truly novel functions. Yet, phylogenetic dissimilarity is often a barrier for HGT.
360 Phylogenetic distant species are less likely to share the homology necessary for processes
361 that rely in homologous recombination, phages typically cannot infect dissimilar hosts, and
362 genetic networks are not always capable of accommodating novel genes. For instance,
363 resistance to antimicrobial peptides (an ancient immune defense in animals and plants) is
364 pervasive in gut microbiomes, but its transfer across bacterial species is limited due to
365 functional incompatibilities between phylogenetic distant bacteria⁷⁵. On the other hand, highly
366 diverse communities can lead to adaptation of the MGEs to different strains, which in turn
367 could lead to modified rates of HGT in their (new, or old) bacterial strains. Phages were
368 observed to expand the range of bacteria they can infect to novel species through low-efficient
369 infection of intermediary bacterial species in the gastrointestinal environment⁷⁶. Such phage
370 host-range expansion can lead to phage-mediated genetic exchange across more divergent
371 bacterial strains. Hence, even if phylogenetic diversity can be a barrier to genetic exchange,
372 some MGEs are capable to overcome (or adapt to profit from) such differences in host-
373 associated communities.

374

375 Another potential major influence of host-associated environments in HGT is the heterogeneity
376 of certain abiotic factors, such as pH, oxygen, nutrient levels or temperature. These factors
377 can vary both spatially (e.g. along the intestinal tract) and temporally, and they affect the
378 physiological state of bacteria. Hence, they can directly impact the efficiency of genetic
379 exchange. For instance, the activation of the competence apparatus necessary for
380 transformation is upregulated at stationary phase in *B. subtilis*, but inhibited in *Streptococcus*
381 *pneumoniae* under the same physiological conditions⁷⁷. The mammalian gut environment has
382 also been shown to interfere with the expression of bacterial genes that mediate the efficiency
383 of phage infection⁷⁸. Induction of prophages in the gastrointestinal environment can also be
384 driven by host-specific factors, such as bile salts or ethanolamine, a byproduct of eukaryotic
385 cell death⁷⁹. Yet, in most cases, it is not always clear how MGEs are affected by host-
386 associated abiotic factors. For instance, out of 13 plasmids tested *in vivo* and *in vitro*, the

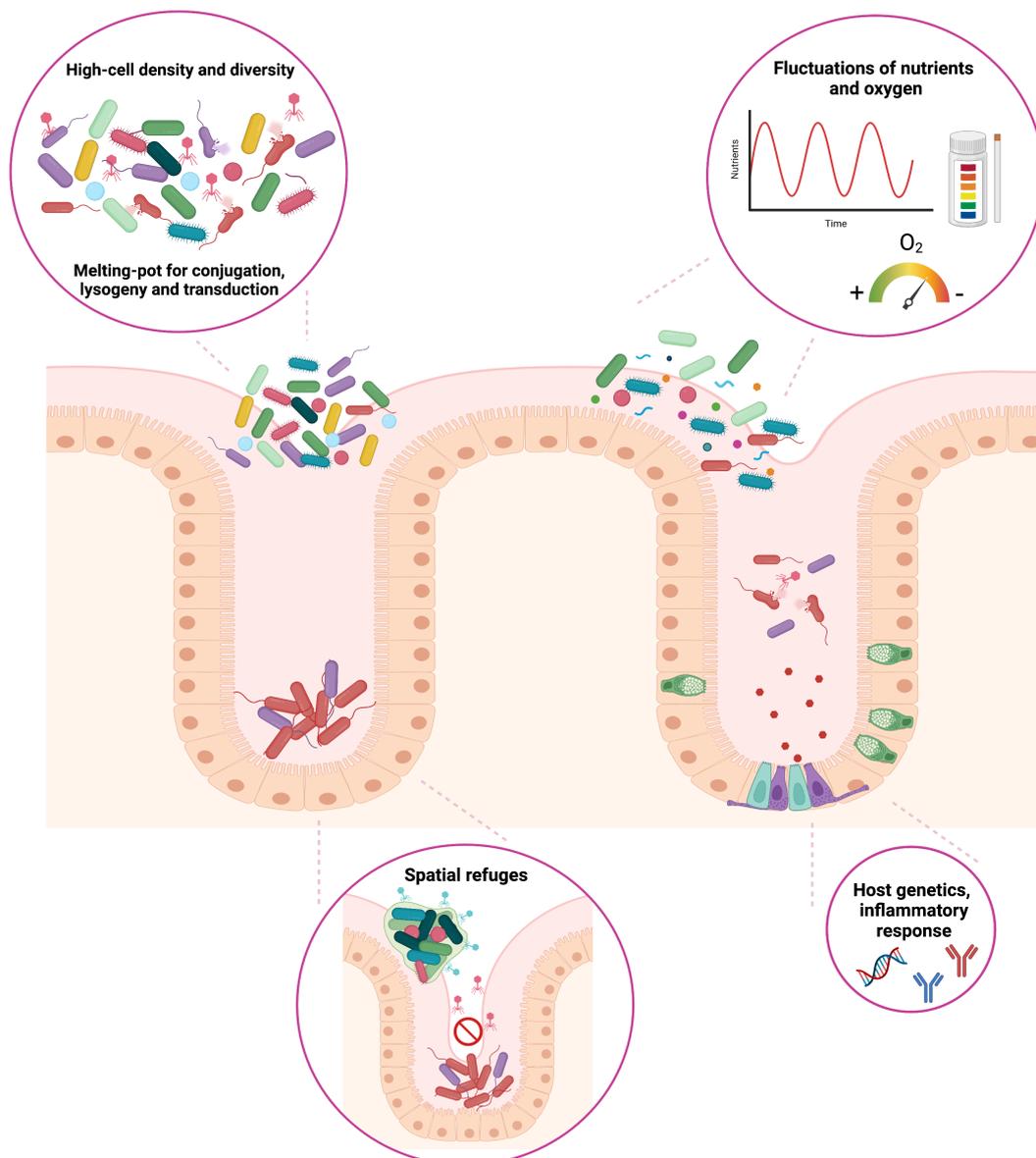
387 conjugative plasmid TP114, was the only one with increased activity at low oxygen levels⁶⁴.
388 Low oxygen is a characteristic of anaerobic conditions in the mammalian gut and in other host-
389 associated environments, which suggests that host-specific physiological conditions can be
390 more favourable to certain plasmids. Fluctuations in the type and availability of nutrients can
391 also lead to an enrichment of different species over time. This has the potential to increase
392 the activity of MGEs encoded by the favored bacteria. The scarcity of nutrients *in vitro* was
393 shown to favor the scavenging of exogenous DNA as a source of energy, thus increasing
394 rates of transformation⁸⁰. Furthermore, several food additives were shown to increase
395 conjugation rates of plasmids carrying antibiotic resistant genes⁸¹. This suggests that HGT in
396 microbiomes can be partially modulated by the dietary intake of their animal hosts.

397

398

399

400



401
 402 **Figure 2. The gut environment as an example of the host-associated factors that can influence horizontal**
 403 **gene transfer.** Schematic representation of different factors that can influence HGT in the mammalian gut. The
 404 high bacterial cell density potentiates close contacts between phylogenetically diverse species, which can drive
 405 high rates of conjugation and multiple contacts with viral (phage) particles. However, the spatially structured gut
 406 can also generate niches that serve as refuges against phage infection, and consequently transduction and
 407 lysogenization. Fluctuations in several abiotic factors, such as nutrients, temperature or pH can have an impact in
 408 the physiology of bacteria, and thus modulate the rates of genetic transfers. Moreover, the host itself, including its
 409 immune system, can shape both microbiome composition and the frequency and types of HGT.

410
 411 Within a host, bacteria are also exposed to many different molecules, either produced or
 412 ingested by the host, or produced by other members of the microbial community. These
 413 molecules can function as inducers of SOS-responses, which can, in turn, potentiate HGT⁸².
 414 Some studies have shown that the inflammatory response induced by the gut colonization of
 415 a pathogenic *Salmonella enterica* serovar Typhimurium may lead to blooms of other

416 enterobacterial strains, and to trafficking of conjugative plasmids between pathogenic and
417 commensal bacteria⁶⁵. However, other studies have proposed that the genetics of the animal
418 host and the composition of the microbiota itself have a higher impact on conjugation rates
419 than the inflammatory process⁶⁶. Host inflammatory responses, e.g., colitis also alters phage
420 communities in the gut⁸³, and promotes phage transfer between different *Salmonella*
421 species⁸⁴. Finally, HGT can also be affected by molecules produced by other bacteria present
422 within the microbiomes. This was recently shown to be the case of colibactin, a genotoxic
423 molecule produced by several *Enterobacteriaceae* in the mammalian gut. Colibactin can
424 induce SOS-responses, and consequently induce the excision of prophages from both gram-
425 positive and gram-negative bacteria that are in close proximity to the colibactin-producing
426 cell⁸⁵. These molecules impact the activity of their MGEs but also affect the composition of
427 microbial communities as whole. Hence, the overall consequences of toxins and stress-
428 inducing agents in HGT processes requires further study.

429

430 The host-associated environmental factors described here are both diverse and temporally
431 dynamic, and possess distinct features that might strongly (albeit differently) impact both the
432 mechanisms and the rates of HGT. This poses an important challenge when comparing HGT
433 processes between *in vivo* and *in vitro* experimental conditions. Critically, it also raises
434 difficulties in identifying the different factors involved in HGT, which is key to truly grasp the
435 impact of this mode of microbial evolution within hosts. Some studies, which we discuss below,
436 have made important leaps towards disentangling the factors of HGT *in vivo*.

437

438

439 Detecting and quantifying within-host HGT in the act

440

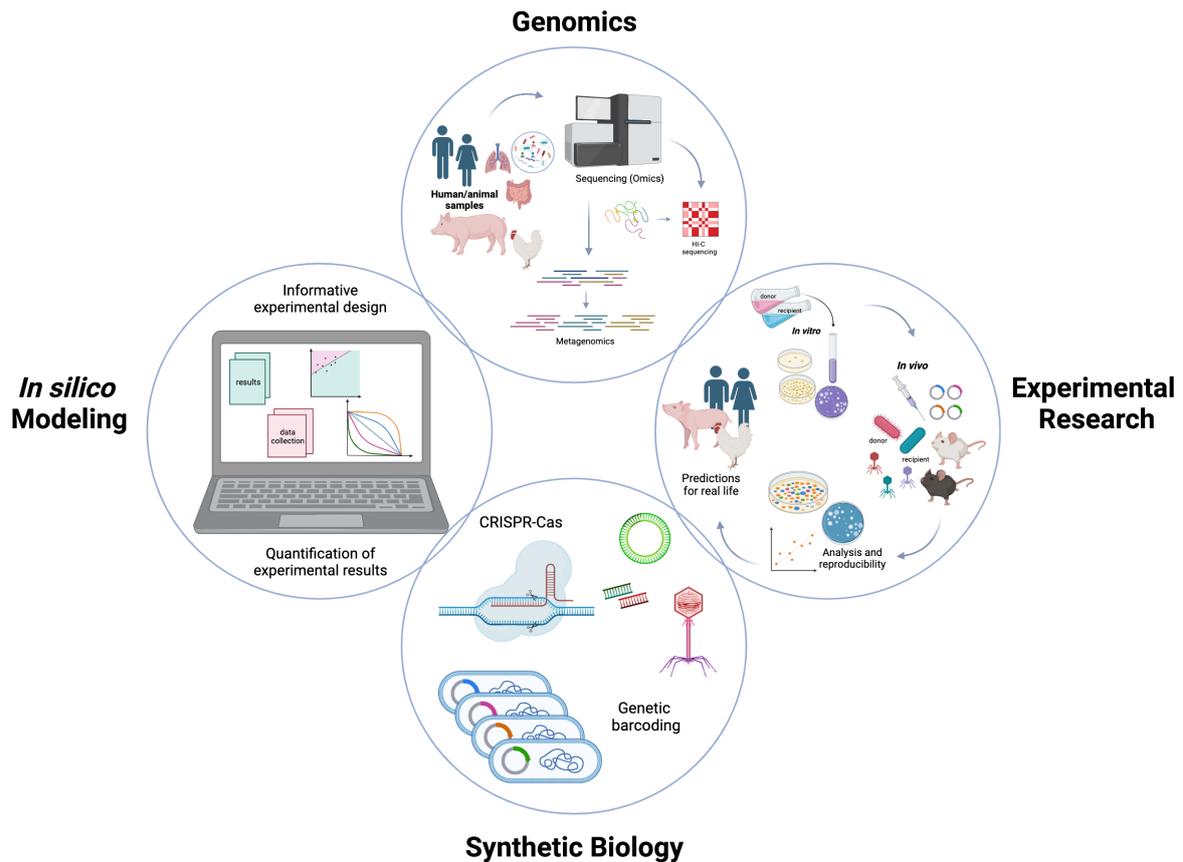
441 The ecological and evolutionary dynamics of multiple interacting, co-evolving MGEs pose a
442 challenge to understand the role of HGT within complex host environments. Yet, other, more
443 fundamental questions pose significant challenges too. In particular, the “simple” matter of
444 how to quantify the rates of the different HGT mechanisms is crucial. This is generally not an
445 easy task. Besides the frequency of transfer of a gene, or an MGE, other parameters need to
446 be considered, such as the impact on fitness of the transferred DNA for both the bacteria and
447 their mobile elements. Indeed, it is likely that many HGT events are deleterious, neutral or only
448 slightly beneficial, and never increase sufficiently in frequency to be detected by current
449 methods⁸⁶. Other HGT events may reach detectable frequencies, but might be transient (e.g.,
450 advantageous only in an ephemeral environment) and only remain in the population for a

451 limited number of generations, thus remaining unaccounted for. In addition, the observation of
452 a highly successful HGT event (i.e., that quickly spreads in a population) can be caused either
453 by a high intrinsic rate of transfer or by a high fitness advantage of what is transferred, even if
454 the actual rate of transfer might be low. Disentangling these factors *in vitro* is already not trivial;
455 to do so within the ecological and physiological complexity of within-host environments is
456 remarkably challenging. But there are different strategies that have been used to isolate these
457 evolutionary components and to track the dynamics of HGT (**Figure 3**), either by itself, or in
458 relation to mutational processes.

459

460 Several methods have now been developed, each with different strengths and weakness, that
461 allow to identify MGEs, to determine their genomic context and to quantify HGT events in
462 metagenomes (reviewed in^{16,87}). Furthermore, specific experimental designs both *in vitro* and
463 *in vivo*, coupled with the use of mathematical models to fit the data, have facilitated the
464 dissociation of the two main components involved in HGT, fitness and transmission. In the
465 case of conjugation, the integration of mathematical models and experiments led to the
466 estimation of plasmid transfer rates between a donor and a recipient *E. coli* strain, both in the
467 guts of mice and also in continuous-flow cultures (that simulated the gut abiotic
468 environment)⁸⁸. Plasmids were observed to spread at a lower rate in the gut of mice, relative
469 to *in vitro* conditions, not due to inefficient or inhibited conjugation, but instead because the
470 donor strain had a lower growth rate *in vivo*, relative to the other competitors within the
471 microbiome. This somehow old study exemplifies that in any event of transfer, several
472 concurrent processes (e.g., bacterial competition, plasmid segregation and the fitness costs
473 associated with plasmid carriage) can impact the rates and outcomes of HGT.

474



475
476
477
478
479
480
481
482
483
484
485
486

Figure 3. Multidisciplinary and integrative approaches are key to understand horizontal gene transfer in within-host environments. The study of HGT has benefited from the integration of different approaches, represented here by the four different circles. Developments in genomics (e.g., next-generation sequencing or comparative genomics) allows the detection and analysis of HGT-mediated changes at a large genomic scale. Novel experimental approaches allow comparison between *in vitro* and *in vivo* results, and an exploration of the dynamics of HGT in complex host associated environments. This is also driven by novel methods in synthetic biology, that allow experimental designs with an appreciable diversity in the genetics of bacteria. Finally, *in silico* models have been key in exploring conditions difficult to test experimentally, provide testable hypotheses for conditions that are amenable to experimentation, and help in the quantification of relevant evolutionary parameters (e.g., the frequency of conjugation or the rate of adaptation of phages).

487
488
489
490
491
492
493
494
495

Experimental evolution has been a powerful method to study evolutionary dynamics of bacterial lineages in real time as they adapt to different *in vitro* environments. More recently, it has been used to catch HGT events in the act in host-associated microbiomes, specifically in the mammalian gastrointestinal tract. In this methodology it is typical to have a cohort of genetical identical animal hosts (e.g., germ-free mice, devoid of any microbes) that are colonized with one or more specific bacterial strains (e.g., that differ in their potential for HGT). The eco-evolutionary dynamics of these strains are then followed in real time. These models were recently used to address the costs and benefits of prophage carriage in the mouse gut, using a well-known bacteria/phage pair: *E. coli* and its (pro)phage λ . By colonizing mice with

496 two strains of *E. coli*, differing solely in the presence/absence of the prophage, it was found
497 that there is rapid phage transfer from the lysogenic to the susceptible strain in the
498 gastrointestinal tract⁸⁹. Additional colonizations with derivatives strains (either phage resistant,
499 or with non-inducible phages) allowed to quantify the cost of carrying the phage. This cost was
500 found to differ substantially across environments: *E. coli* lysogens were found to have a much
501 higher induction rate in this *in vivo* system than in classic *in vitro* growth media. This study
502 also revealed a high probability of lysogenization in the gut (~19%) and also facilitated the
503 detection of rare events of phage gene capture within the first days of gut colonization⁸⁹. In a
504 different study, prophage carriage was found to also incur a fitness cost in *Lactobacillus*
505 *reuteri*, but to provide a competitive fitness advantage for lysogens when competing with non-
506 lysogens in the gut of germ-free mice⁹⁰. Moreover, phage production in a *L. reuteri* strain with
507 two active prophages differed along the digestive tract, i.e. being higher in the cecum and
508 colon relative to the small intestine. Importantly, phage production in the gut was additionally
509 identified to be modulated by the amount of fructose, an abundant sugar in western diets⁹¹.
510 This is an important example of how the dynamics of MGEs (temperate phages) differ across
511 host-associated environments (cecum, colon and small intestine), and can be modulated by
512 abiotic factors (nutrients).

513

514 A more recent study in mice with a rich microbiota composition, demonstrated how phage-
515 driven HGT can shape both the short⁹² and long-term evolution⁹³ of an *E. coli* strain colonizing
516 the gut. The colonizing strain was the target of rapid lysogenization by two prophages present
517 within another *E. coli* strain, a natural resident of the mouse gut microbiome. These lysogenic
518 events, which occurred in less than two weeks, were key for the successful and stable
519 colonization of the invader *E. coli*. Afterwards, the newly evolved lysogens accumulated
520 several mutations over thousands of generations of evolution in the gut. Some of these
521 mutations reduced the induction rate of the acquired prophages, i.e., it was possible to follow
522 the process of domestication of the prophages in real time. The rate of spontaneous prophage
523 induction in evolved clones was similar to that measured in the original, resident *E. coli* strain,
524 for which the induction rate did not change along time⁹³. These results are consistent with the
525 theoretical prediction of a non-zero spontaneous induction rate leading to an optimal strategy
526 in gut commensal bacteria⁹⁴. Importantly, this study highlighted how HGT and mutational
527 processes are intertwined, and revealed that two very distinct modes of selection can drive
528 the evolution of an invader strain: while in one mode newly emerging mutations increase in
529 frequency but never reach fixation in the population, in the other the successive fixation of
530 mutations strongly interplays with the HGT promoted by phages and plasmids. Another
531 glimpse of this type of coevolution is provided by endemic infections caused by *Vibrio*
532 *cholerae*, whose outbreaks are modulated by their lytic phages (which are, in turn, modulated

533 by their phage satellites). Bacteria and their phages recovered from the patients' longitudinal
534 sampling has shown that *V. cholerae* isolated from a given time point were susceptible to the
535 phages isolated at the same time point, but resistant to phages from successive time points⁹⁵.
536 The dynamics of phages correlated with fluctuations in SXT encoded in ICEs, which besides
537 conferring AR, also encode anti-phage defenses, providing a remarkable link of both types of
538 resistances in a single MGE.

539

540 Some studies also attempted to discern how the genetic background of an animal host affects
541 the dynamics of HGT in its microbiome, with somewhat different conclusions across the
542 different studies^{65,66}. This suggests a need for systematization in approaches, which should
543 combine both different animal genetic backgrounds and diverse bacterial strains, to better
544 understand how (e.g.) transconjugants can rapidly and stably colonize their hosts. The costs
545 of carrying a plasmid for the fitness of a bacterial strain is also key to understand the
546 maintenance of transconjugants in host-associated environments. For instance, the fitness
547 effect of a conjugative plasmid that encodes for AR was shown to be on average negative in
548 the absence of antibiotics, across a large collection of strains of *E. coli* and *Klebsiella* that
549 colonize human gut microbiomes⁹⁶. However, there was extensive epistasis of its fitness
550 effects across the strains tested, and remarkably, in some strains, the plasmid was even
551 beneficial in the absence of antibiotics. This highlights that extensive variation in the fitness
552 effects of a plasmid across strains may help explain its maintenance within some microbiomes
553 but not others.

554

555 Technological advances in genomic engineering, comparative genomics and metagenomics
556 also contributed to study the diversity and distribution of MGEs, as well as the frequency of
557 HGT, within hosts. Recent developments in sequencing, such as split-read insertion
558 detection⁹⁷, long-read sequencing (e.g., Nanopore⁹⁸) or single-cell sequencing⁹⁹ can be
559 harnessed to improve the detection of HGT events. Other recent approaches were developed
560 more specifically for this purpose. For instance, a CRISPR system was engineered to detect
561 and record plasmid and phage transfer events in an *E. coli*¹⁰⁰. The metagenomic analysis of
562 within-host communities has allowed comparing the microbiomes and phageomes of healthy
563 and unhealthy patients, revealing that the phage gut diversity is typically severely decreased
564 in the latter¹⁰¹. Furthermore, a massive analysis of thousands of human gut metagenomes
565 identified new families of Caudovirales phages, as well as new potential mechanisms of
566 phage-bacteria interactions *in vivo*, that are encoded by phages. This includes Diversity-
567 Generating Retroelements (DGR), lipid biosynthesis, directed alterations of the bacterial cell-
568 wall, as well as catalases and iron sequestering enzymes¹⁰², all of which can be adaptive when
569 transferred (e.g. through lysogeny) to other bacterial hosts. Such events of capture and

570 transfer of traits by MGEs might be frequent, and it may occur not only within, but also between
571 hosts. A large scale longitudinal genomic and metabolomics analysis of maternal and infant
572 microbiomes identified that infant microbiomes were composed of bacteria that had unique
573 species-metabolite relationships, absent from their mothers' microbiomes. This study
574 suggests an inheritance of microbiome-encoded metabolic traits not due to the transfer of
575 bacteria, but likely due to HGT promoted by inherited MGEs¹⁰³. Circulating MGEs may then
576 be prevalent within hosts, as also highlighted by a metagenomic Hi-C analysis of 10 healthy
577 human gut samples. This study indicated that, while 75% of the detected phage-like
578 sequences are likely dormant prophages, 5% have a high sequence coverage, suggesting
579 that the human gut microbiota has an important fraction of actively replicating phages⁷². The
580 same technology has suggested that HGT occurs frequently in the individual guts of both
581 healthy and neutropenic patients undergoing stem cell transplantation, and that it might involve
582 the exchange of AR genes¹⁰⁴. Conversely, a metagenomic approach that was used to directly
583 address the bacterial content that is carried within phage particles of phages in pig fecal
584 microbiomes demonstrated that phages do not frequently carry or encode AR genes¹⁰⁵.

585

586 The latter studies highlight the controversial role of phages in the transfer of antibiotic
587 resistance genes, a trait that is typically considered to be more efficiently trafficked by
588 plasmids. Given the growing impact of AR, the dissemination of AR genes in MGEs has been,
589 understandably, the focus of a large part of the literature on HGT. Yet, it is important to
590 consider the evolutionary dynamics involved in the mobilization and stability of AR genes in
591 mobile elements, and the role of antibiotics themselves in shaping the rates of HGT.

592

593 HGT in the context of antibiotic resistance

594

595 Antibiotics are some of the most widely used antimicrobials not only in clinical settings but also
596 agriculture, farming and food industry. As resistance to antibiotics became a wide societal
597 problem, particular attention has been dedicated to how such traits disseminate rapidly within
598 and between bacterial populations. MGEs may be key for these dynamics in some bacterial
599 species, but not necessarily in others¹⁰⁶. A recent study, that coupled mathematical modelling
600 with experimental assays in soil communities, demonstrated that microbial communities that
601 encode AR genes in conjugative plasmids are more stable when facing environmental
602 perturbations (e.g., heavy metals or antibiotics), compared to communities where bacteria
603 encode AR within their chromosomes¹⁰⁷. This may partially explain the prevalence of AR

604 genes in plasmids, and why the latter are one of the main culprits for the wide and fast
605 dissemination of AR, particularly in a clinical context¹⁰⁸. Additionally, there is a high abundance
606 of plasmid-encoded genes involved in cellular metabolism and stress, that might underlie the
607 development of AR mechanisms¹⁰⁹. In many cases, the fitness advantage in surviving
608 exposure to antibiotics is so dramatic that even very costly plasmids can be maintained.
609 However, those costs can be mitigated. A recent study observed the acquisition of
610 compensatory mutations in *Enterobacter* isolates that led to the prevalence of a carbapenem-
611 resistance carrying plasmid (pOXA-48) in the gut of hospitalised patients. These
612 compensatory mutations occurred in plasmid-associated fitness genes, resulting in a trade-off
613 between the fitness cost of the plasmid and the level of resistance to carbapenem¹¹⁰. Such
614 compensatory adaptations stabilize AR genes in the populations, and many plasmids that
615 encode such traits are maintained even in the absence of antibiotics¹¹¹. Phages are not
616 thought to be traffickers of AR genes *in vivo*¹⁰⁵, but other studies argue that phages are
617 important vehicles of such genes¹¹². The high efficiency of phage-mediated transfer of AR
618 genes *in vitro* suggests a possible underestimation of these events *in vivo*.

619

620 Antibiotics also have a physiological effect that can impact HGT. Some antibiotics induce
621 SOS-responses, and *in vitro* assays have shown that antibiotics, such as aminoglycosides or
622 fluoroquinolones can trigger competence in *S. pneumoniae* and other species¹¹³⁻¹¹⁵. The effect
623 of antibiotics on conjugation is controversial, despite some studies showing increased rates of
624 conjugation in their presence¹¹⁶. The effect on phages seems to depend on the type of
625 antibiotics. Aminoglycoside antibiotics (such as Kanamycin or Apramycin) were shown to
626 inhibit phage infection and replication¹¹⁷. On the other hand DNA damaging antibiotics (like
627 Mitomycin C or Fluoroquinolones) are well known to stimulate prophage induction in many
628 lysogens, and it has been suggested that some common oral medications may have a similar
629 role¹¹⁸. Hence, a higher prophage induction and excision due to medical drugs might lead to
630 increased rates of lysogeny and transduction. Another typical response from bacteria to
631 antibiotic exposure is the increased production of exopolysaccharides or lipopolysaccharides,
632 that can promote the formation of biofilms¹¹⁹. Biofilms can increase the chances of contacts
633 between bacterial cells, and conjugation rates have been proposed to increase under biofilm
634 formation¹²⁰. On the other hand, while phage propagation and dispersion might be slowed
635 down by sparse, but locally dense, sub-populations, the chances of lysogeny itself may
636 increase, due to a high local multiplicity of infection⁶⁷. Interestingly, conjugation itself can
637 promote the formation of biofilms¹²⁰, highlighting the complex interrelationship between
638 antibiotics, resistance to antibiotics, and mobilization of AR genes.

639

640 CONCLUSION

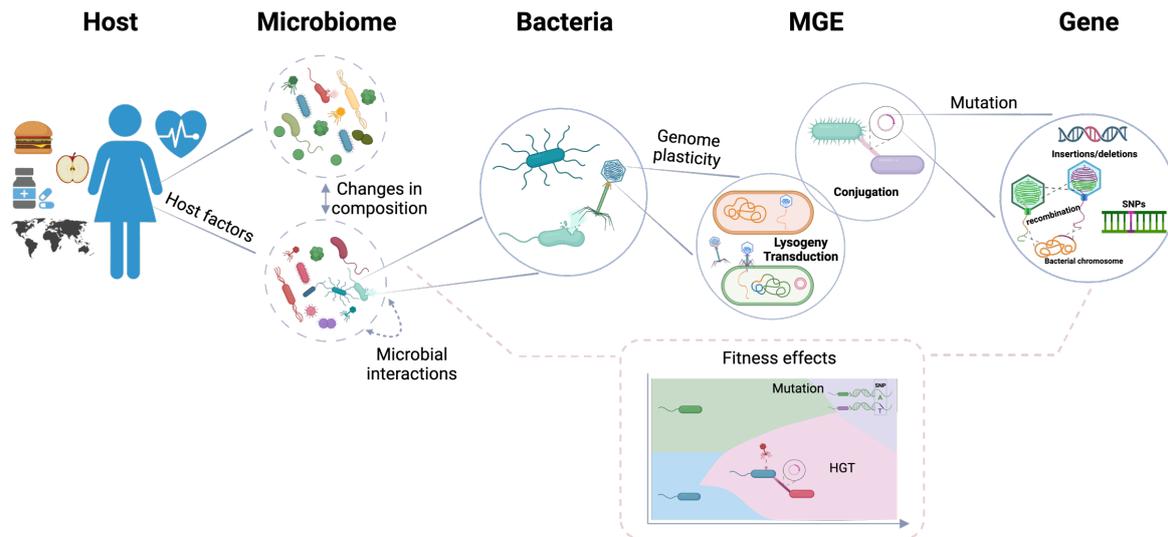
641

642 The study of microbial adaptation, and in particular of the mechanisms that involve the
643 horizontal exchange of DNA, is at an interesting standpoint. On the one hand, the scientific
644 community is, technologically, better suited than ever to detect all sorts of novel bacterial and
645 mobile element variants, and how their frequencies change almost in real time. On the other
646 hand, this deluge of information has uncovered an ever increasingly complex, rapidly evolving
647 network of interactions, that tremendously complicates the quantification of the individual
648 factors involved in HGT. Indeed, although there is an extensive body of highly important *in*
649 *vitro* work in quantifying the rates of HGT, there is still a gap between those observations in
650 simplified environments compared to those faced by bacteria *in vivo*. This is in part due to the
651 challenges posed in measuring these complex but subtle changes across bacteria, in contexts
652 of fluctuating conditions (including a huge variety of stresses), high bacterial diversity or even
653 different intricate processes of adaptation occurring simultaneously.

654

655 There are different levels of selection involved and affected by HGT: from the gene, to the
656 MGE that mobilizes the gene, to the bacterium that receives the MGE, to the microbiome of
657 this recipient, and to the living organism (animal, or plant) that hosts this particular microbial
658 community (**Figure 4**). This means that, in order to uncover the impact of HGT in within-host
659 bacterial evolution, the rates and fitness effects of the mechanisms of horizontal transfer must
660 be understood at different biological and temporal scales, that reflect both environmental and
661 epidemiological conditions. Important care must also be given to uncoupling the relationship
662 between the *rates of transfer* and the *effect on fitness* provided by the MGE and/or the
663 transferred DNA. This relationship is in turn influenced by the different fluctuating conditions
664 endured during host colonization, making a very advantageous trait in a certain ecosystem
665 rapidly shift to deleterious in another environment. Furthermore, the accumulation of mutations
666 subsequent to the HGT event, and how this can both influence and be influenced by HGT
667 itself, is not yet well understood.

668



669

670 **Figure 4. A general view of the different levels, and their interactions, involved in study of within-host**
 671 **horizontal gene transfer.** Understanding HGT *in vivo* requires the consideration of the different scales at which
 672 ecological and evolutionary process occur. The dietary and societal conditions of the host influence the composition
 673 and evolutionary dynamics of their microbiomes. The latter are determined by the interactions (competitive or
 674 cooperative) and genetic exchanges that occur between the bacteria. This, in turn, is determined at the individual
 675 bacterial level, by the combination of its rate of generating mutations, that are vertically inherited, and the rate of
 676 horizontal transfer of multiple MGEs that increase or decrease the bacterial gene repertoires (as exemplified in the
 677 Muller plot shown). Importantly, these same processes (competitive and cooperative interactions, vertical and
 678 horizontal adaptation) affect the MGEs themselves. This creates a complex, multi-layered network of events that,
 679 as a whole, determines rates and effects of HGT within and between animal hosts.

680

681 The multiverse of MGEs, mechanisms of HGT and the context dependent nature of transfer
 682 rates and effects begs for a screening of *in vivo* measurements under conditions where
 683 manipulation of donor-recipient densities can be controlled. In addition, given the complex
 684 interactions between MGEs, new mathematical models with far more events and populations
 685 need to be developed. As more events imply a larger number of parameters, more data is
 686 required to avoid model overfitting. Thus, some ingenious tricks also need to be developed to
 687 capitalize the most on the limited maneuverability of *in vivo* experiments. In this regard the
 688 usage of multi-omics and synthetic biology approaches will be key. In particular, a wider
 689 adoption of barcodes to label several donors and recipient cells, that are then introduced in
 690 natural or synthetic ecosystems at different initial densities may aid in a better quantitative
 691 understanding of the most dominant processes underlying successful HGT events.

692

693 Recent and exciting technological advances, coupled with a growing number of studies that
 694 integrate computational and experimental approaches, have been key in overcoming some of
 695 the challenges involved in detecting and quantifying within-host HGT. Given the current state

696 of affairs on such a fundamental evolutionary process with tremendous planetary-wide
697 consequences, particularly regarding human health, we expect that other studies will follow
698 suit. This will hopefully lead to the establishment of a substantial amount of research where
699 disciplines such as eco-evolutionary modeling, animal experimentation and synthetic biology
700 integrate to close the knowledge gap.

701

702 ACKNOWLEDGEMENTS

703

704 The authors thank Eugen Pfeifer for comments and suggestions on an earlier version of this
705 manuscript. Illustrations were created with BioRender.com.

706

707 Conflict of interest

708

709 The authors declare that they do not have any conflict of interest.

710

711

712

713 REFERENCES

714

- 715 1. Johnson, K.V.-A., and Foster, K.R. (2018). Why does the microbiome affect behaviour?
716 Nat. Rev. Microbiol. 16, 647–655. 10.1038/s41579-018-0014-3.
- 717 2. Henry, L.P., Bruijning, M., Forsberg, S.K.G., and Ayroles, J.F. (2021). The microbiome
718 extends host evolutionary potential. Nat. Commun. 12, 5141. 10.1038/s41467-021-
719 25315-x.
- 720 3. Yang, Y., Nguyen, M., Khetrapal, V., Sonnert, N.D., Martin, A.L., Chen, H., Kriegel, M.A.,
721 and Palm, N.W. (2022). Within-host evolution of a gut pathobiont facilitates liver
722 translocation. Nature 607, 563–570. 10.1038/s41586-022-04949-x.
- 723 4. Haudiquet, M., de Sousa, J.M., Touchon, M., and Rocha, E.P.C. (2022). Selfish,
724 promiscuous and sometimes useful: how mobile genetic elements drive horizontal gene
725 transfer in microbial populations. Philos. Trans. R. Soc. B Biol. Sci. 377, 20210234.
726 10.1098/rstb.2021.0234.
- 727 5. Ramiro, R.S., Durão, P., Bank, C., and Gordo, I. (2020). Low mutational load and high
728 mutation rate variation in gut commensal bacteria. PLoS Biol. 18, e3000617.
729 10.1371/journal.pbio.3000617.
- 730 6. Vulić, M., Dionisio, F., Taddei, F., and Radman, M. (1997). Molecular keys to speciation:
731 DNA polymorphism and the control of genetic exchange in enterobacteria. Proc. Natl.
732 Acad. Sci. 94, 9763–9767. 10.1073/pnas.94.18.9763.
- 733 7. Rathmann, I., Förster, M., Yüksel, M., Horst, L., Petrungaro, G., Bollenbach, T., and
734 Maier, B. (2023). Distribution of fitness effects of cross-species transformation reveals
735 potential for fast adaptive evolution. ISME J. 17, 130–139. 10.1038/s41396-022-01325-
736 5.
- 737 8. Redfield, R.J., Schrag, M.R., and Dean, A.M. (1997). The evolution of bacterial
738 transformation: sex with poor relations. Genetics 146, 27–38.
739 10.1093/genetics/146.1.27.
- 740 9. Durieux, I., Ginevra, C., Attaiech, L., Picq, K., Juan, P.-A., Jarraud, S., and Charpentier, X.
741 (2019). Diverse conjugative elements silence natural transformation in *Legionella*
742 species. Proc. Natl. Acad. Sci. 116, 18613–18618. 10.1073/pnas.1909374116.
- 743 10. Hamilton, H.L., and Dillard, J.P. (2006). Natural transformation of *Neisseria*
744 *gonorrhoeae* : from DNA donation to homologous recombination: Natural
745 transformation of *Neisseria gonorrhoeae*. Mol. Microbiol. 59, 376–385. 10.1111/j.1365-
746 2958.2005.04964.x.
- 747 11. Barany, F., and Kahn, M.E. (1985). Comparison of transformation mechanisms of
748 *Haemophilus parainfluenzae* and *Haemophilus influenzae*. J. Bacteriol. 161, 72–79.
749 10.1128/jb.161.1.72-79.1985.

- 750 12. Pimentel, Z.T., and Zhang, Y. (2018). Evolution of the Natural Transformation Protein,
751 ComEC, in Bacteria. *Front. Microbiol.* *9*, 2980. 10.3389/fmicb.2018.02980.
- 752 13. Toyofuku, M., Nomura, N., and Eberl, L. (2019). Types and origins of bacterial membrane
753 vesicles. *Nat. Rev. Microbiol.* *17*, 13–24. 10.1038/s41579-018-0112-2.
- 754 14. Dell’Annunziata, F., Dell’Aversana, C., Doti, N., Donadio, G., Dal Piaz, F., Izzo, V., De
755 Filippis, A., Galdiero, M., Altucci, L., Boccia, G., et al. (2021). Outer Membrane Vesicles
756 Derived from *Klebsiella pneumoniae* Are a Driving Force for Horizontal Gene Transfer.
757 *Int. J. Mol. Sci.* *22*, 8732. 10.3390/ijms22168732.
- 758 15. Klieve, A.V., Yokoyama, M.T., Forster, R.J., Ouwerkerk, D., Bain, P.A., and Mawhinney,
759 E.L. (2005). Naturally Occurring DNA Transfer System Associated with Membrane
760 Vesicles in Cellulolytic *Ruminococcus* spp. of Ruminal Origin. *Appl. Environ. Microbiol.*
761 *71*, 4248–4253. 10.1128/AEM.71.8.4248-4253.2005.
- 762 16. Brito, I.L. (2021). Examining horizontal gene transfer in microbial communities. *Nat. Rev.*
763 *Microbiol.* *19*, 442–453. 10.1038/s41579-021-00534-7.
- 764 17. Khedkar, S., Smyshlyayev, G., Letunic, I., Maistrenko, O.M., Coelho, L.P., Orakov, A.,
765 Forslund, S.K., Hildebrand, F., Luetge, M., Schmidt, T.S.B., et al. (2022). Landscape of
766 mobile genetic elements and their antibiotic resistance cargo in prokaryotic genomes.
767 *Nucleic Acids Res.* *50*, 3155–3168. 10.1093/nar/gkac163.
- 768 18. Pant, A., Bag, S., Saha, B., Verma, J., Kumar, P., Banerjee, S., Kumar, B., Kumar, Y.,
769 Desigamani, A., Maiti, S., et al. (2020). Molecular insights into the genome dynamics and
770 interactions between core and acquired genomes of *Vibrio cholerae*. *Proc. Natl. Acad.*
771 *Sci.* *117*, 23762–23773. 10.1073/pnas.2006283117.
- 772 19. Castillo, D., Kauffman, K., Hussain, F., Kalatzis, P., Rørbo, N., Polz, M.F., and Middelboe,
773 M. (2018). Widespread distribution of prophage-encoded virulence factors in marine
774 *Vibrio* communities. *Sci. Rep.* *8*, 9973. 10.1038/s41598-018-28326-9.
- 775 20. Touchon, M., Bernheim, A., and Rocha, E.P. (2016). Genetic and life-history traits
776 associated with the distribution of prophages in bacteria. *ISME J.* *10*, 2744–2754.
777 10.1038/ismej.2016.47.
- 778 21. Kim, M.-S., and Bae, J.-W. (2018). Lysogeny is prevalent and widely distributed in the
779 murine gut microbiota. *ISME J.* *12*, 1127–1141. 10.1038/s41396-018-0061-9.
- 780 22. Chiang, Y.N., Penadés, J.R., and Chen, J. (2019). Genetic transduction by phages and
781 chromosomal islands: The new and noncanonical. *PLOS Pathog.* *15*, e1007878.
782 10.1371/journal.ppat.1007878.
- 783 23. Chen, J., Quiles-Puchalt, N., Chiang, Y.N., Bacigalupe, R., Fillol-Salom, A., Chee, M.S.J.,
784 Fitzgerald, J.R., and Penadés, J.R. (2018). Genome hypermobility by lateral transduction.
785 *Science* *362*, 207–212. 10.1126/science.aat5867.

- 786 24. Fillol-Salom, A., Alsaadi, A., Sousa, J.A.M. de, Zhong, L., Foster, K.R., Rocha, E.P.C.,
787 Penadés, J.R., Ingmer, H., and Haaber, J. (2019). Bacteriophages benefit from
788 generalized transduction. *PLOS Pathog.* *15*, e1007888. 10.1371/journal.ppat.1007888.
- 789 25. Schmieger, H. (1972). Phage P22-mutants with increased or decreased transduction
790 abilities. *Mol. Gen. Genet. MGG* *119*, 75–88. 10.1007/BF00270447.
- 791 26. Li, X.-Y., Lachnit, T., Fraune, S., Bosch, T.C.G., Traulsen, A., and Sieber, M. (2017).
792 Temperate phages as self-replicating weapons in bacterial competition. *J. R. Soc.*
793 *Interface* *14*, 20170563. 10.1098/rsif.2017.0563.
- 794 27. Davies, E.V., James, C.E., Kukavica-Ibrulj, I., Levesque, R.C., Brockhurst, M.A., and
795 Winstanley, C. (2016). Temperate phages enhance pathogen fitness in chronic lung
796 infection. *ISME J.* *10*, 2553–2555. 10.1038/ismej.2016.51.
- 797 28. de Sousa, J.A.M., Buffet, A., Haudiquet, M., Rocha, E.P.C., and Rendueles, O. (2020).
798 Modular prophage interactions driven by capsule serotype select for capsule loss under
799 phage predation. *ISME J.* *14*, 2980–2996. 10.1038/s41396-020-0726-z.
- 800 29. Haaber, J., Leisner, J.J., Cohn, M.T., Catalan-Moreno, A., Nielsen, J.B., Westh, H.,
801 Penadés, J.R., and Ingmer, H. (2016). Bacterial viruses enable their host to acquire
802 antibiotic resistance genes from neighbouring cells. *Nat. Commun.* *7*, 13333.
803 10.1038/ncomms13333.
- 804 30. Bertozzi Silva, J., Storms, Z., and Sauvageau, D. (2016). Host receptors for bacteriophage
805 adsorption. *FEMS Microbiol. Lett.* *363*, fnw002. 10.1093/femsle/fnw002.
- 806 31. Kauffman, K.M., Chang, W.K., Brown, J.M., Hussain, F.A., Yang, J., Polz, M.F., and Kelly, L.
807 (2022). Resolving the structure of phage-bacteria interactions in the context of natural
808 diversity. *Nat. Commun.* *13*, 372. 10.1038/s41467-021-27583-z.
- 809 32. Jain, A., and Srivastava, P. (2013). Broad host range plasmids. *FEMS Microbiol. Lett.* *348*,
810 87–96. 10.1111/1574-6968.12241.
- 811 33. Adelberg, E.A., and Pittard, J. (1965). CHROMOSOME TRANSFER IN BACTERIAL
812 CONJUGATION. *Bacteriol. Rev.* *29*, 161–172. 10.1128/br.29.2.161-172.1965.
- 813 34. Hall, J.P.J., Wright, R.C.T., Harrison, E., Muddiman, K.J., Wood, A.J., Paterson, S., and
814 Brockhurst, M.A. (2021). Plasmid fitness costs are caused by specific genetic conflicts
815 enabling resolution by compensatory mutation. *PLOS Biol.* *19*, e3001225.
816 10.1371/journal.pbio.3001225.
- 817 35. Ibarra-Chávez, R., Hansen, M.F., Pinilla-Redondo, R., Seed, K.D., and Trivedi, U. (2021).
818 Phage satellites and their emerging applications in biotechnology. *FEMS Microbiol. Rev.*
819 *45*, fuab031. 10.1093/femsre/fuab031.
- 820 36. de Sousa, J.A.M., Fillol-Salom, A., Penadés, J.R., and Rocha, E.P.C. (2023). Identification
821 and characterization of thousands of bacteriophage satellites across bacteria. *Nucleic*
822 *Acids Res.*, gkad123. 10.1093/nar/gkad123.

- 823 37. Moura de Sousa, J.A., and Rocha, E.P.C. (2022). To catch a hijacker: abundance,
824 evolution and genetic diversity of P4-like bacteriophage satellites. *Philos. Trans. R. Soc. B*
825 *Biol. Sci.* 377, 20200475. 10.1098/rstb.2020.0475.
- 826 38. Fillol-Salom, A., Martínez-Rubio, R., Abdulrahman, R.F., Chen, J., Davies, R., and Penadés,
827 J.R. (2018). Phage-inducible chromosomal islands are ubiquitous within the bacterial
828 universe. *ISME J.* 12, 2114–2128. 10.1038/s41396-018-0156-3.
- 829 39. Alqurainy, N., Miguel-Romero, L., Moura de Sousa, J., Chen, J., Rocha, E.P.C., Fillol-
830 Salom, A., and Penades, J.R. (2022). A widespread family of phage-inducible
831 chromosomal islands only steals bacteriophage tails to spread in nature.
832 10.1101/2022.09.08.507074.
- 833 40. O’Hara, B.J., Barth, Z.K., McKitterick, A.C., and Seed, K.D. (2017). A highly specific phage
834 defense system is a conserved feature of the *Vibrio cholerae* mobilome. *PLOS Genet.* 13,
835 e1006838. 10.1371/journal.pgen.1006838.
- 836 41. Penadés, J.R., and Christie, G.E. (2015). The Phage-Inducible Chromosomal Islands: A
837 Family of Highly Evolved Molecular Parasites. *Annu. Rev. Virol.* 2, 181–201.
838 10.1146/annurev-virology-031413-085446.
- 839 42. Rousset, F., Depardieu, F., Miele, S., Dowding, J., Laval, A.-L., Lieberman, E., Garry, D.,
840 Rocha, E.P.C., Bernheim, A., and Bikard, D. (2022). Phages and their satellites encode
841 hotspots of antiviral systems. *Cell Host Microbe* 30, 740-753.e5.
842 10.1016/j.chom.2022.02.018.
- 843 43. Fillol-Salom, A., Rostøl, J.T., Ojiogu, A.D., Chen, J., Douce, G., Humphrey, S., and
844 Penadés, J.R. (2022). Bacteriophages benefit from mobilizing pathogenicity islands
845 encoding immune systems against competitors. *Cell* 185, 3248-3262.e20.
846 10.1016/j.cell.2022.07.014.
- 847 44. Coluzzi, C., Garcillán-Barcia, M.P., de la Cruz, F., and Rocha, E.P.C. (2022). Evolution of
848 Plasmid Mobility: Origin and Fate of Conjugative and Nonconjugative Plasmids. *Mol.*
849 *Biol. Evol.* 39, msac115. 10.1093/molbev/msac115.
- 850 45. Ares-Arroyo, M., Coluzzi, C., and P.C. Rocha, E. (2022). Origins of transfer establish
851 networks of functional dependencies for plasmid transfer by conjugation. *Nucleic Acids*
852 *Res.*, gkac1079. 10.1093/nar/gkac1079.
- 853 46. Guédon, G., Libante, V., Coluzzi, C., Payot, S., and Leblond-Bourget, N. (2017). The
854 Obscure World of Integrative and Mobilizable Elements, Highly Widespread Elements
855 that Pirate Bacterial Conjugative Systems. *Genes* 8, 337. 10.3390/genes8110337.
- 856 47. Pfeifer, E., Moura de Sousa, J.A., Touchon, M., and Rocha, E.P.C. (2021). Bacteria have
857 numerous distinctive groups of phage–plasmids with conserved phage and variable
858 plasmid gene repertoires. *Nucleic Acids Res.* 49, 2655–2673. 10.1093/nar/gkab064.

- 859 48. Pfeifer, E., Bonnin, R.A., and Rocha, E.P.C. (2022). Phage-Plasmids Spread Antibiotic
860 Resistance Genes through Infection and Lysogenic Conversion. *mBio* *13*, e01851-22.
861 10.1128/mbio.01851-22.
- 862 49. Rocha, E.P.C., and Bikard, D. (2022). Microbial defenses against mobile genetic elements
863 and viruses: Who defends whom from what? *PLoS Biol.* *20*, e3001514.
864 10.1371/journal.pbio.3001514.
- 865 50. Tesson, F., Hervé, A., Mordret, E., Touchon, M., d’Humières, C., Cury, J., and Bernheim,
866 A. (2022). Systematic and quantitative view of the antiviral arsenal of prokaryotes. *Nat.*
867 *Commun.* *13*, 2561. 10.1038/s41467-022-30269-9.
- 868 51. Cheng, K., Wilkinson, M., Chaban, Y., and Wigley, D.B. (2020). A conformational switch in
869 response to Chi converts RecBCD from phage destruction to DNA repair. *Nat. Struct.*
870 *Mol. Biol.* *27*, 71–77. 10.1038/s41594-019-0355-2.
- 871 52. Oliveira, P.H., Touchon, M., and Rocha, E.P.C. (2014). The interplay of restriction-
872 modification systems with mobile genetic elements and their prokaryotic hosts. *Nucleic*
873 *Acids Res.* *42*, 10618–10631. 10.1093/nar/gku734.
- 874 53. Pinilla-Redondo, R., Russel, J., Mayo-Muñoz, D., Shah, S.A., Garrett, R.A., Nesme, J.,
875 Madsen, J.S., Fineran, P.C., and Sørensen, S.J. (2022). CRISPR-Cas systems are
876 widespread accessory elements across bacterial and archaeal plasmids. *Nucleic Acids*
877 *Res.* *50*, 4315–4328. 10.1093/nar/gkab859.
- 878 54. Touchon, M., Perrin, A., de Sousa, J.A.M., Vangchhia, B., Burn, S., O’Brien, C.L., Denamur,
879 E., Gordon, D., and Rocha, E.P. (2020). Phylogenetic background and habitat drive the
880 genetic diversification of *Escherichia coli*. *PLOS Genet.* *16*, e1008866.
881 10.1371/journal.pgen.1008866.
- 882 55. Horne, T., Orr, V.T., and Hall, J.P. (2023). How do interactions between mobile genetic
883 elements affect horizontal gene transfer? *Curr. Opin. Microbiol.* *73*, 102282.
884 10.1016/j.mib.2023.102282.
- 885 56. Lindqvist, B.H., Dehò, G., and Calendar, R. (1993). Mechanisms of genome propagation
886 and helper exploitation by satellite phage P4. *Microbiol. Rev.* *57*, 683–702.
- 887 57. Chen, J., Ram, G., Penadés, J.R., Brown, S., and Novick, R.P. (2015). Pathogenicity Island-
888 Directed Transfer of Unlinked Chromosomal Virulence Genes. *Mol. Cell* *57*, 138–149.
889 10.1016/j.molcel.2014.11.011.
- 890 58. Humphrey, S., San Millán, Á., Toll-Riera, M., Connolly, J., Flor-Duro, A., Chen, J., Ubeda,
891 C., MacLean, R.C., and Penadés, J.R. (2021). Staphylococcal phages and pathogenicity
892 islands drive plasmid evolution. *Nat. Commun.* *12*, 5845. 10.1038/s41467-021-26101-5.
- 893 59. Brady, A., Quiles-Puchalt, N., Gallego del Sol, F., Zamora-Caballero, S., Felipe-Ruíz, A.,
894 Val-Calvo, J., Meijer, W.J.J., Marina, A., and Penadés, J.R. (2021). The arbitrium system
895 controls prophage induction. *Curr. Biol.* *31*, 5037-5045.e3. 10.1016/j.cub.2021.08.072.

- 896 60. Erez, Z., Steinberger-Levy, I., Shamir, M., Doron, S., Stokar-Avihail, A., Peleg, Y.,
897 Melamed, S., Leavitt, A., Savidor, A., Albeck, S., et al. (2017). Communication between
898 viruses guides lysis–lysogeny decisions. *Nature* 541, 488–493. 10.1038/nature21049.
- 899 61. Haag, A.F., Podkowik, M., Ibarra-Chávez, R., Gallego del Sol, F., Ram, G., Chen, J., Marina,
900 A., Novick, R.P., and Penadés, J.R. (2021). A regulatory cascade controls *Staphylococcus*
901 *aureus* pathogenicity island activation. *Nat. Microbiol.* 6, 1300–1308. 10.1038/s41564-
902 021-00956-2.
- 903 62. Groussin, M., Poyet, M., Sistiaga, A., Kearney, S.M., Moniz, K., Noel, M., Hooker, J.,
904 Gibbons, S.M., Segurel, L., Froment, A., et al. (2021). Elevated rates of horizontal gene
905 transfer in the industrialized human microbiome. *Cell* 184, 2053-2067.e18.
906 10.1016/j.cell.2021.02.052.
- 907 63. Sharp, C., and Foster, K.R. (2022). Host control and the evolution of cooperation in host
908 microbiomes. *Nat. Commun.* 13, 3567. 10.1038/s41467-022-30971-8.
- 909 64. Neil, K., Allard, N., Grenier, F., Burrus, V., and Rodrigue, S. (2020). Highly efficient gene
910 transfer in the mouse gut microbiota is enabled by the IncI2 conjugative plasmid TP114.
911 *Commun. Biol.* 3, 523. 10.1038/s42003-020-01253-0.
- 912 65. Stecher, B., Denzler, R., Maier, L., Bernet, F., Sanders, M.J., Pickard, D.J., Barthel, M.,
913 Westendorf, A.M., Krogfelt, K.A., Walker, A.W., et al. (2012). Gut inflammation can
914 boost horizontal gene transfer between pathogenic and commensal *Enterobacteriaceae*.
915 *Proc. Natl. Acad. Sci.* 109, 1269–1274. 10.1073/pnas.1113246109.
- 916 66. Ott, L.C., Stromberg, Z.R., Redweik, G.A.J., Wannemuehler, M.J., and Mellata, M. (2020).
917 Mouse Genetic Background Affects Transfer of an Antibiotic Resistance Plasmid in the
918 Gastrointestinal Tract. *mSphere* 5, e00847-19. 10.1128/mSphere.00847-19.
- 919 67. Sousa, J.A.M. de, and Rocha, E.P.C. (2019). Environmental structure drives resistance to
920 phages and antibiotics during phage therapy and to invading lysogens during
921 colonisation. *Sci. Rep.* 9, 3149. 10.1038/s41598-019-39773-3.
- 922 68. Shkoporov, A.N., Stockdale, S.R., Lavelle, A., Kondova, I., Heuston, C., Upadrasta, A.,
923 Khokhlova, E.V., van der Kamp, I., Ouwering, B., Draper, L.A., et al. (2022). Viral
924 biogeography of the mammalian gut and parenchymal organs. *Nat. Microbiol.* 7, 1301–
925 1311. 10.1038/s41564-022-01178-w.
- 926 69. Lourenço, M., Chaffringeon, L., Lamy-Besnier, Q., Pédrón, T., Campagne, P., Eberl, C.,
927 Bérard, M., Stecher, B., Debarbieux, L., and De Sordi, L. (2020). The Spatial
928 Heterogeneity of the Gut Limits Predation and Fosters Coexistence of Bacteria and
929 Bacteriophages. *Cell Host Microbe* 28, 390-401.e5. 10.1016/j.chom.2020.06.002.
- 930 70. Dewald-Wang, E.A., Parr, N., Tiley, K., Lee, A., and Koskella, B. (2022). Multiyear Time-
931 Shift Study of Bacteria and Phage Dynamics in the Phyllosphere. *Am. Nat.* 199, 126–140.
932 10.1086/717181.

- 933 71. Shkoporov, A.N., Clooney, A.G., Sutton, T.D.S., Ryan, F.J., Daly, K.M., Nolan, J.A.,
934 McDonnell, S.A., Khokhlova, E.V., Draper, L.A., Forde, A., et al. (2019). The Human Gut
935 Virome Is Highly Diverse, Stable, and Individual Specific. *Cell Host Microbe* 26, 527-
936 541.e5. 10.1016/j.chom.2019.09.009.
- 937 72. Marbouty, M., Thierry, A., Millot, G.A., and Koszul, R. (2021). MetaHiC phage-bacteria
938 infection network reveals active cycling phages of the healthy human gut. *eLife* 10,
939 e60608. 10.7554/eLife.60608.
- 940 73. Human Microbiome Project Consortium (2012). Structure, function and diversity of the
941 healthy human microbiome. *Nature* 486, 207–214. 10.1038/nature11234.
- 942 74. Conwill, A., Kuan, A.C., Damerla, R., Poret, A.J., Baker, J.S., Tripp, A.D., Alm, E.J., and
943 Lieberman, T.D. (2022). Anatomy promotes neutral coexistence of strains in the human
944 skin microbiome. *Cell Host Microbe* 30, 171-182.e7. 10.1016/j.chom.2021.12.007.
- 945 75. Kintsès, B., Méhi, O., Ari, E., Számel, M., Györkei, Á., Jangir, P.K., Nagy, I., Pál, F., Fekete,
946 G., Tengölics, R., et al. (2018). Phylogenetic barriers to horizontal transfer of
947 antimicrobial peptide resistance genes in the human gut microbiota. *Nat. Microbiol.* 4,
948 447–458. 10.1038/s41564-018-0313-5.
- 949 76. De Sordi, L., Khanna, V., and Debarbieux, L. (2017). The Gut Microbiota Facilitates Drifts
950 in the Genetic Diversity and Infectivity of Bacterial Viruses. *Cell Host Microbe* 22, 801-
951 808.e3. 10.1016/j.chom.2017.10.010.
- 952 77. Claverys, J.-P., Prudhomme, M., and Martin, B. (2006). Induction of competence
953 regulons as a general response to stress in gram-positive bacteria. *Annu. Rev. Microbiol.*
954 60, 451–475. 10.1146/annurev.micro.60.080805.142139.
- 955 78. Lourenço, M., Chaffringeon, L., Lamy-Besnier, Q., Titécat, M., Pédrón, T., Sismeiro, O.,
956 Legendre, R., Varet, H., Coppée, J.-Y., Bérard, M., et al. (2022). The gut environment
957 regulates bacterial gene expression which modulates susceptibility to bacteriophage
958 infection. *Cell Host Microbe* 30, 556-569.e5. 10.1016/j.chom.2022.03.014.
- 959 79. Henrot, C., and Petit, M. (2022). Signals triggering prophage induction in the gut
960 microbiota. *Mol. Microbiol.* 118, 494–502. 10.1111/mmi.14983.
- 961 80. Sinha, S., Mell, J., and Redfield, R. (2013). The availability of purine nucleotides regulates
962 natural competence by controlling translation of the competence activator Sxy. *Mol.*
963 *Microbiol.* 88, 1106–1119. 10.1111/mmi.12245.
- 964 81. Yu, Z., Wang, Y., Lu, J., Bond, P.L., and Guo, J. (2021). Nonnutritive sweeteners can
965 promote the dissemination of antibiotic resistance through conjugative gene transfer.
966 *ISME J.* 15, 2117–2130. 10.1038/s41396-021-00909-x.
- 967 82. Beaber, J.W., Hochhut, B., and Waldor, M.K. (2004). SOS response promotes horizontal
968 dissemination of antibiotic resistance genes. *Nature* 427, 72–74. 10.1038/nature02241.

- 969 83. Duerkop, B.A., Kleiner, M., Paez-Espino, D., Zhu, W., Bushnell, B., Hassell, B., Winter,
970 S.E., Kyrpides, N.C., and Hooper, L.V. (2018). Murine colitis reveals a disease-associated
971 bacteriophage community. *Nat. Microbiol.* *3*, 1023–1031. 10.1038/s41564-018-0210-y.
- 972 84. Diard, M., Bakkeren, E., Cornuault, J.K., Moor, K., Hausmann, A., Sellin, M.E., Loverdo, C.,
973 Aertsen, A., Ackermann, M., De Paepe, M., et al. (2017). Inflammation boosts
974 bacteriophage transfer between *Salmonella* spp. *Science* *355*, 1211–1215.
975 10.1126/science.aaf8451.
- 976 85. Silpe, J.E., Wong, J.W.H., Owen, S.V., Baym, M., and Balskus, E.P. (2022). The bacterial
977 toxin colibactin triggers prophage induction. *Nature* *603*, 315–320. 10.1038/s41586-022-
978 04444-3.
- 979 86. Woods, L.C., Gorrell, R.J., Taylor, F., Connallon, T., Kwok, T., and McDonald, M.J. (2020).
980 Horizontal gene transfer potentiates adaptation by reducing selective constraints on the
981 spread of genetic variation. *Proc. Natl. Acad. Sci.* *117*, 26868–26875.
982 10.1073/pnas.2005331117.
- 983 87. Douglas, G.M., and Langille, M.G.I. (2019). Current and Promising Approaches to Identify
984 Horizontal Gene Transfer Events in Metagenomes. *Genome Biol. Evol.* *11*, 2750–2766.
985 10.1093/gbe/evz184.
- 986 88. Freter, R., Brickner, H., Fekete, J., Vickerman, M.M., and Carey, K.E. (1983). Survival and
987 Implantation of *Escherichia coli* in the Intestinal Tract. *Infect. Immun.* *39*, 686–703.
988 10.1128/iai.39.2.686-703.1983.
- 989 89. De Paepe, M., Tournier, L., Moncaut, E., Son, O., Langella, P., and Petit, M.-A. (2016).
990 Carriage of λ Latent Virus Is Costly for Its Bacterial Host due to Frequent Reactivation in
991 Monoxenic Mouse Intestine. *PLOS Genet.* *12*, e1005861. 10.1371/journal.pgen.1005861.
- 992 90. Oh, J.-H., Lin, X.B., Zhang, S., Tollenaar, S.L., Özçam, M., Dunphy, C., Walter, J., and van
993 Pijkeren, J.-P. (2019). Prophages in *Lactobacillus reuteri* Are Associated with Fitness
994 Trade-Offs but Can Increase Competitiveness in the Gut Ecosystem. *Appl. Environ.*
995 *Microbiol.* *86*, e01922-19. 10.1128/AEM.01922-19.
- 996 91. Oh, J.-H., Alexander, L.M., Pan, M., Schueler, K.L., Keller, M.P., Attie, A.D., Walter, J., and
997 van Pijkeren, J.-P. (2019). Dietary Fructose and Microbiota-Derived Short-Chain Fatty
998 Acids Promote Bacteriophage Production in the Gut Symbiont *Lactobacillus reuteri*. *Cell*
999 *Host Microbe* *25*, 273-284.e6. 10.1016/j.chom.2018.11.016.
- 1000 92. Frazão, N., Sousa, A., Lässig, M., and Gordo, I. (2019). Horizontal gene transfer overrides
1001 mutation in *Escherichia coli* colonizing the mammalian gut. *Proc. Natl. Acad. Sci.* *116*,
1002 17906–17915. 10.1073/pnas.1906958116.
- 1003 93. Frazão, N., Konrad, A., Amicone, M., Seixas, E., Güleresi, D., Lässig, M., and Gordo, I.
1004 (2022). Two modes of evolution shape bacterial strain diversity in the mammalian gut
1005 for thousands of generations. *Nat. Commun.* *13*, 5604. 10.1038/s41467-022-33412-8.

- 1006 94. Cortes, M.G., Krog, J., and Balázsi, G. (2019). Optimality of the spontaneous prophage
1007 induction rate. *J. Theor. Biol.* *483*, 110005. 10.1016/j.jtbi.2019.110005.
- 1008 95. LeGault, K., Hays, S.G., Angermeyer, A., McKitterick, A.C., Johura, F., Sultana, M., Alam,
1009 M., and Seed, K.D. (2020). Temporal Shifts in Antibiotic Resistance Elements Govern
1010 Virus-Pathogen Conflicts (Microbiology) 10.1101/2020.12.16.423150.
- 1011 96. Alonso-del Valle, A., León-Sampedro, R., Rodríguez-Beltrán, J., DelaFuente, J.,
1012 Hernández-García, M., Ruiz-Garbajosa, P., Cantón, R., Peña-Miller, R., and San Millán, A.
1013 (2021). Variability of plasmid fitness effects contributes to plasmid persistence in
1014 bacterial communities. *Nat. Commun.* *12*, 2653. 10.1038/s41467-021-22849-y.
- 1015 97. Jiang, X., Hall, A.B., Xavier, R.J., and Alm, E.J. (2019). Comprehensive analysis of
1016 chromosomal mobile genetic elements in the gut microbiome reveals phylum-level
1017 niche-adaptive gene pools. *PLOS ONE* *14*, e0223680. 10.1371/journal.pone.0223680.
- 1018 98. Peter, S., Bosio, M., Gross, C., Bezdán, D., Gutierrez, J., Oberhettinger, P., Liese, J., Vogel,
1019 W., Dörfel, D., Berger, L., et al. (2020). Tracking of Antibiotic Resistance Transfer and
1020 Rapid Plasmid Evolution in a Hospital Setting by Nanopore Sequencing. *mSphere* *5*,
1021 e00525-20. 10.1128/mSphere.00525-20.
- 1022 99. Labonté, J.M., Field, E.K., Lau, M., Chivian, D., Van Heerden, E., Wommack, K.E., Kieft,
1023 T.L., Onstott, T.C., and Stepanauskas, R. (2015). Single cell genomics indicates horizontal
1024 gene transfer and viral infections in a deep subsurface Firmicutes population. *Front.*
1025 *Microbiol.* *6*. 10.3389/fmicb.2015.00349.
- 1026 100. Munck, C., Sheth, R.U., Freedberg, D.E., and Wang, H.H. (2020). Recording mobile
1027 DNA in the gut microbiota using an *Escherichia coli* CRISPR-Cas spacer acquisition
1028 platform. *Nat. Commun.* *11*, 95. 10.1038/s41467-019-14012-5.
- 1029 101. Manrique, P., Bolduc, B., Walk, S.T., van der Oost, J., de Vos, W.M., and Young, M.J.
1030 (2016). Healthy human gut phageome. *Proc. Natl. Acad. Sci.* *113*, 10400–10405.
1031 10.1073/pnas.1601060113.
- 1032 102. Benler, S., Yutin, N., Antipov, D., Rayko, M., Shmakov, S., Gussow, A.B., Pevzner, P.,
1033 and Koonin, E.V. (2021). Thousands of previously unknown phages discovered in whole-
1034 community human gut metagenomes. *Microbiome* *9*, 78. 10.1186/s40168-021-01017-w.
- 1035 103. Vatanen, T., Jabbar, K.S., Ruohtula, T., Honkanen, J., Avila-Pacheco, J., Siljander, H.,
1036 Stražar, M., Oikarinen, S., Hyöty, H., Ilonen, J., et al. (2022). Mobile genetic elements
1037 from the maternal microbiome shape infant gut microbial assembly and metabolism.
1038 *Cell* *185*, 4921-4936.e15. 10.1016/j.cell.2022.11.023.
- 1039 104. Kent, A.G., Vill, A.C., Shi, Q., Satlin, M.J., and Brito, I.L. (2020). Widespread transfer of
1040 mobile antibiotic resistance genes within individual gut microbiomes revealed through
1041 bacterial Hi-C. *Nat. Commun.* *11*, 4379. 10.1038/s41467-020-18164-7.
- 1042 105. Billaud, M., Lamy-Besnier, Q., Lossouarn, J., Moncaut, E., Dion, M.B., Moineau, S.,
1043 Traoré, F., Le Chatelier, E., Denis, C., Estelle, J., et al. (2021). Analysis of viromes and

- 1044 microbiomes from pig fecal samples reveals that phages and prophages rarely carry
1045 antibiotic resistance genes. *ISME Commun.* *1*, 55. 10.1038/s43705-021-00054-8.
- 1046 106. Lehtinen, S., Chewapreecha, C., Lees, J., Hanage, W.P., Lipsitch, M., Croucher, N.J.,
1047 Bentley, S.D., Turner, P., Fraser, C., and Mostowy, R.J. (2020). Horizontal gene transfer
1048 rate is not the primary determinant of observed antibiotic resistance frequencies in
1049 *Streptococcus pneumoniae*. *Sci. Adv.* *6*, eaaz6137. 10.1126/sciadv.aaz6137.
- 1050 107. Coyte, K.Z., Stevenson, C., Knight, C.G., Harrison, E., Hall, J.P.J., and Brockhurst, M.A.
1051 (2022). Horizontal gene transfer and ecological interactions jointly control microbiome
1052 stability. *PLoS Biol.* *20*, e3001847. 10.1371/journal.pbio.3001847.
- 1053 108. San Millan, A. (2018). Evolution of Plasmid-Mediated Antibiotic Resistance in the
1054 Clinical Context. *Trends Microbiol.* *26*, 978–985. 10.1016/j.tim.2018.06.007.
- 1055 109. Palomino, A., Gewurz, D., DeVine, L., Zajmi, U., Morales, J., Abu-Rumman, F., Smith,
1056 R.P., and Lopatkin, A.J. (2022). Metabolic genes on conjugative plasmids are highly
1057 prevalent in *Escherichia coli* and can protect against antibiotic treatment. *ISME J.*
1058 10.1038/s41396-022-01329-1.
- 1059 110. DelaFuente, J., Toribio-Celestino, L., Santos-Lopez, A., León-Sampedro, R., Alonso-Del
1060 Valle, A., Costas, C., Hernández-García, M., Cui, L., Rodríguez-Beltrán, J., Bikard, D., et al.
1061 (2022). Within-patient evolution of plasmid-mediated antimicrobial resistance. *Nat.*
1062 *Ecol. Evol.* *6*, 1980–1991. 10.1038/s41559-022-01908-7.
- 1063 111. Lopatkin, A.J., Meredith, H.R., Srimani, J.K., Pfeiffer, C., Durrett, R., and You, L.
1064 (2017). Persistence and reversal of plasmid-mediated antibiotic resistance. *Nat.*
1065 *Commun.* *8*, 1689. 10.1038/s41467-017-01532-1.
- 1066 112. Fernández-Orth, D., Miró, E., Brown-Jaque, M., Rodríguez-Rubio, L., Espinal, P.,
1067 Rodríguez-Navarro, J., González-López, J.J., Muniesa, M., and Navarro, F. (2019). Faecal
1068 phageome of healthy individuals: presence of antibiotic resistance genes and variations
1069 caused by ciprofloxacin treatment. *J. Antimicrob. Chemother.* *74*, 854–864.
1070 10.1093/jac/dky540.
- 1071 113. Wang, Y., Lu, J., Engelstädter, J., Zhang, S., Ding, P., Mao, L., Yuan, Z., Bond, P.L., and
1072 Guo, J. (2020). Non-antibiotic pharmaceuticals enhance the transmission of exogenous
1073 antibiotic resistance genes through bacterial transformation. *ISME J.* *14*, 2179–2196.
1074 10.1038/s41396-020-0679-2.
- 1075 114. Charpentier, X., Polard, P., and Claverys, J.-P. (2012). Induction of competence for
1076 genetic transformation by antibiotics: convergent evolution of stress responses in
1077 distant bacterial species lacking SOS? *Curr. Opin. Microbiol.* *15*, 570–576.
1078 10.1016/j.mib.2012.08.001.
- 1079 115. Prudhomme, M., Attaiech, L., Sanchez, G., Martin, B., and Claverys, J.-P. (2006).
1080 Antibiotic stress induces genetic transformability in the human pathogen *Streptococcus*
1081 *pneumoniae*. *Science* *313*, 89–92. 10.1126/science.1127912.

- 1082 116. Lopatkin, A.J., Huang, S., Smith, R.P., Srimani, J.K., Sysoeva, T.A., Bewick, S., Karig,
1083 D.K., and You, L. (2016). Antibiotics as a selective driver for conjugation dynamics. *Nat.*
1084 *Microbiol.* *1*, 16044. 10.1038/nmicrobiol.2016.44.
- 1085 117. Kever, L., Hardy, A., Luthe, T., Hünnefeld, M., Gätgens, C., Milke, L., Wiechert, J.,
1086 Wittmann, J., Moraru, C., Marienhagen, J., et al. (2022). Aminoglycoside Antibiotics
1087 Inhibit Phage Infection by Blocking an Early Step of the Infection Cycle. *mBio* *13*, e00783-
1088 22. 10.1128/mbio.00783-22.
- 1089 118. Sutcliffe, S.G., Shamash, M., Hynes, A.P., and Maurice, C.F. (2021). Common Oral
1090 Medications Lead to Prophage Induction in Bacterial Isolates from the Human Gut.
1091 *Viruses* *13*, 455. 10.3390/v13030455.
- 1092 119. Ghigo, J.M. (2001). Natural conjugative plasmids induce bacterial biofilm
1093 development. *Nature* *412*, 442–445. 10.1038/35086581.
- 1094 120. Sheppard, R.J., Beddis, A.E., and Barraclough, T.G. (2020). The role of hosts, plasmids
1095 and environment in determining plasmid transfer rates: A meta-analysis. *Plasmid* *108*,
1096 102489. 10.1016/j.plasmid.2020.102489.
- 1097
- 1098
- 1099