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1 **Environmental vibrio phage-bacteria interaction networks reflect the genetic structure**
2 **of host populations**

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22

23 **Originality-Significance Statement.** Cross-infection of phage isolates against a panel of
24 bacterial isolates is a widely used tool to assess key parameters of phage ecology and
25 evolution. The taxonomy and spatiotemporal scale over which the isolates are collected are
26 known to strongly influence infection outcome. Here, we show that differences in the
27 population structure of the host are critical for understanding phage host range and phage load
28 in the environment.

29 **ABSTRACT**

30 Phages depend on their bacterial host to replicate. The habitat, density and genetic diversity of
31 host populations are therefore key factors in phage ecology, but our ability to explore their
32 biology depends on the isolation of a diverse and representative collection of phages from
33 different sources. Here, we compared two populations of marine bacterial hosts and their
34 phages collected during a time series sampling program in an oyster farm. The population of
35 *Vibrio crassostreae*, a species associated specifically to oysters, was genetically structured
36 into clades of near clonal strains, leading to the isolation of closely related phages forming
37 large modules in phage-bacterial infection networks. For *Vibrio chagasii*, which blooms in the
38 water column, a lower number of closely related hosts and a higher diversity of isolated
39 phages resulted in small modules in the phage-bacterial infection network. Over time, phage
40 load was correlated with *V. chagasii* abundance, indicating a role of host blooms in driving
41 phage abundance. Genetic experiments further demonstrated that these phage blooms can
42 generate epigenetic and genetic variability that can counteract host defense systems. These
43 results highlight the importance of considering both the environmental dynamics as well as
44 the genetic structure of the host when interpreting phage-bacteria networks.
45

46 INTRODUCTION

47 Phages, the viral predators of bacteria, constitute the most abundant and diverse biological
48 entities in the ocean and are thought to play a significant role in controlling the composition
49 of microbial communities (Brum and Sullivan 2015, Breitbart, Bonnain et al. 2018). As
50 obligate parasites, phages depend on their bacterial hosts to thrive in the environment and
51 therefore the habitat, density and genetic diversity of host populations are expected to be key
52 determinants of phage ecology. Phage ecology encompasses the breadth of host strains a
53 phage is capable of infecting (host range), as well as adsorption, production and dispersion
54 efficiency in the ecosystem (phage-predator load). The ecology of phages in marine
55 environments remains poorly understood, but such knowledge is important for understanding
56 how they control the expansion of bacterial populations in healthy natural ecosystems and for
57 devising phage applications in disease ridden aquaculture settings (Nobrega, Costa et al.
58 2015, Gordillo Altamirano and Barr 2019, Kortright, Chan et al. 2019).

59 The farming of Pacific oysters is a prime example of an aquaculture setting susceptible to
60 disease, with the *Vibrionaceae* (hereafter referred to as “vibrios”) being the best described
61 bacteria associated with oyster diseases (Le Roux, Wegner et al. 2015). The ecology,
62 diversity, and population structure of vibrios have been extensively studied (reviewed in
63 (Cordero and Polz 2014, Le Roux, Wegner et al. 2016). Despite their very broad genomic
64 diversity, vibrios fall into well-defined phylogenetic clusters that are specifically associated to
65 organic particles or host organisms, or correspond to free-living forms in the water column
66 (Hunt, David et al. 2008, Szabo, Preheim et al. 2012). These phylogenetic clusters represent
67 distinct populations with respect to gene flow (Shapiro, Friedman et al. 2012, Hehemann,
68 Arevalo et al. 2016) and interactions with other organisms (social/behavioural networks)
69 (Cordero, Ventouras et al. 2012, Cordero, Wildschutte et al. 2012, Yawata, Cordero et al.
70 2014, Hehemann, Arevalo et al. 2016) and are predominantly congruent with described *Vibrio*
71 species (Preheim, Timberlake et al. 2011), although with finer evolutionary divergence
72 (Shapiro, Friedman et al. 2012). Following this finer distinction, here we define “populations”
73 as corresponding to members of a vibrio species isolated from the same location.

74 By investigating the disease ecology of vibrios in an oyster farming area, we showed that the
75 onset of disease in oysters is associated with progressive replacement of diverse benign
76 colonizers by members of a virulent population, assigned to *V. crassostreae* (Lemire,
77 Goudenège et al. 2015). Although genetically diverse, most members of this population can

78 cause the disease. *V. crassostreae* was abundant in diseased animals and nearly absent in the
79 surrounding seawater, suggesting that its primary habitat is the oyster (Bruto, James et al.
80 2017). The distribution of *V. crassostreae* in the tissues and circulatory system of oysters
81 revealed a positive association with the hemolymph (blood), supporting the conclusion that
82 this population is adapted to overcoming the immune system of the oysters. This was later
83 confirmed by the identification of virulence factors involved in hemocyte (immune cell)
84 cytotoxicity (Rubio, Oyanedel et al. 2019, Piel, Bruto et al. 2020).

85 An important consequence of the population framework outlined above is that it allows
86 analysis of environmental dynamics of populations, e.g. blooms and busts triggered by
87 specific ecological conditions. We observed that vibrio populations are highly dynamic in the
88 natural ecosystem (Bruto, James et al. 2017), presumably in part due to predation by phages
89 (Piel, Bruto et al. 2022). We isolated a large collection of *V. crassostreae* and vibriophage
90 strains from an oyster farm during a five month time-series sampling program. Cross-
91 infection experiments of roughly 82,000 host-phage pairs revealed a low rate of lytic
92 interaction (2.2%), with interconnected groups of phages and hosts (or “modules”). These
93 modules involved genomic clusters of phages (taxonomically assigned to the genus rank) that
94 adsorb on distinct phylogenetic clades within the *V. crassostreae* species, suggesting the
95 existence of clade-specific receptor(s) and genus-specific receptor binding protein(s).

96 It is unclear, however, whether the structure of phage-bacteria infection networks in large
97 modules is a specific characteristic of *V. crassostreae* or a general feature of phage-vibrio
98 interactions. A study exploring diverse populations of vibrios and their phages revealed that
99 singleton modules of interaction (one phage infecting only one strain) were prevalent,
100 highlighting a remarkably narrow host range of phages with respect to their local hosts in the
101 environment (Kauffman, Chang et al. 2022). Only a few larger modules were described
102 involving 1) broader host range phages that infect strains from diverse populations, and 2)
103 diverse phages that exclusively infect *V. breoganii*, an algae specialist (Corzett, Elsherbini et
104 al. 2018). This suggested that divergence in bacterial ecology, for example the divergence
105 between host-associated and free-living lifestyles, can be reflected in the topography of the
106 interaction network and host range of the phages (Kauffman, Chang et al. 2022).

107 To further investigate the connection between ecology, genetic diversity and infection
108 networks, here we compare the structure of phage-bacteria interactions involving *V.*
109 *crassostreae* and *V. chagasii*. *V. chagasii* was chosen because previous data suggest that its

110 habitat and lifestyle diverge from those of *V. crassostreae*, although the two populations can
111 co-infect the same animal (Bruto, James et al. 2017). Firstly, the relative abundance of *V.*
112 *chagasii* in oysters has been reported to resemble that in surrounding seawater. Secondly, this
113 population preferentially occurred in the free-living (1- 0.2 μm) fraction of seawater and
114 oyster digestive glands, where food particles are concentrated in filter feeding oysters
115 (Froelich and Noble 2014). The presence of *V. chagasii* in the digestive gland is thus likely
116 due to passive transfer by filter-feeding and this population might rather bloom in seawater.
117 To directly compare *V. crassostreae* and *V. chagasi*, we analysed the dynamics of *V. chagasii*
118 and their vibriophages in the same time series previously used to describe the *V. crassostreae*-
119 phage infection network (Piel, Bruto et al. 2022). We combined cultivation, comparative
120 genomics and molecular genetics to analyze a large collection of bacterial isolates and their
121 phages. This allowed determination of crucial parameters of phage ecology by addressing
122 how different host ecologies can feed back on genetic diversity and the structure of the
123 resulting host-phage interaction networks.

124 **RESULTS AND DISCUSSION**

125 **Phage load correlates with *V. chagasii* frequency**

126 We sampled vibrios from juvenile oysters deployed in an oyster farm (Bay of Brest, France)
127 and from the surrounding seawater on 57 dates over five months, including a period with
128 mortality events (Piel, Bruto et al. 2022). On each sampling date, total vibrios from oysters
129 and seawater were isolated on selective media. Of the 5268 randomly picked colonies, 252
130 isolates were assigned to *V. chagasii* by sequencing and analysis of the gene encoding the
131 DNA gyrase subunit B. A total of 144 *V. chagasii* isolates were obtained from the 1-0.2 μm
132 (free-living) seawater fraction, while the remaining 108 isolates were obtained from oyster
133 tissues (Table 1). *V. chagasii* was detected later than *V. crassostreae* and persisted after the
134 disease outbreak, suggesting that a role in oyster disease is unlikely (Fig. 1a).

135 Each of the 252 *V. chagasii* isolates were used as "bait" to detect co-occurring lytic phages
136 from both 1) seawater viral concentrate, and 2) oyster tissues (504 combinations tested).
137 Phage infection was assessed by plaque formation in soft agar overlays of host lawns mixed
138 with the viral source (see Methods). Of the 252 strains, 113 (44.8%) were plaque positive, 59
139 of which were isolated from seawater and 54 from oysters (Table 1). The vast majority of
140 plaque-forming units (PFUs) were obtained using seawater concentrate as the viral source

141 (2023 PFUs from seawater vs 35 PFUs from oysters) which could be expected considering
142 that viruses from seawater were concentrated 1000X. The number of phages infecting *V.*
143 *chagasii* in seawater was estimated to range from 50 L⁻¹ (1 PFU observed on the plate using
144 20mL-seawater equivalents of viral concentrate 1000X) to more than 5.10³ L⁻¹ depending on
145 the strain and date. Phage load was significantly correlated with *V. chagasii* frequency in both
146 isolation fractions (Fig. 1b), consistent with a role of host blooms in driving phage abundance
147 (Kauffman, Chang et al. 2022). Twice as many PFUs were obtained using vibrios isolated
148 from oysters as prey (1347 vs 676 when using vibrio from seawater, Table 1), but this
149 difference was driven by the high proportion of *V. chagasii* isolated from oysters on August
150 25th (30/108; 30%), with 29 out of every 30 isolates being plaque positive leading to a total of
151 1088 PFUs. By comparison, the ratio of plaque positive to total number of hosts (18/194,
152 9.2%) and the number of plaques per host were significantly lower for *V. crassostreae* in both
153 isolation fractions (Table 1). Detection of only 47 PFUs from seawater viral concentrates vs
154 2023 PFUs for *V. chagasii* confirmed the scarcity of *V. crassostreae* in the seawater. It is
155 noteworthy that during oyster disease at least 50% of oysters die rapidly and massive numbers
156 of *V. crassostreae* will be released into the water column. The low number of PFUs in
157 seawater suggests that vibrios from this population rapidly colonize new animals rather than
158 remaining in the water column.

159 **Structure of phage-bacteria interactions reflects genomic diversity and phylogeny**

160 To investigate the host range of phages isolated using *V. chagasii*, we purified one phage
161 from each plaque positive host to obtain a final set of 95 phage isolates. These phages were
162 tested against 252 *V. chagasii* isolates from the time series and 106 representative members of
163 other vibrio populations previously isolated in the same location (Bruto, James et al. 2017).
164 This cross-infection assay revealed highly specific interactions between phages and bacteria
165 since only 1047 of the 34010 tested interactions (3%) were positive (Fig.S1), with no phages
166 infecting members of other populations.

167 We next sequenced and assembled the genomes of selected host strains (Table S1). A total of
168 136 representatives were chosen by excluding the *V. chagasii* isolates with identical *gyrB*
169 sequences and identical patterns of susceptibility in the cross-infection assays. The core
170 genome phylogeny and pairwise ANI values revealed striking differences between hosts (Fig.
171 2a). In *V. crassostreae* the genetic distance was low within clades and large between clades

172 (Fig. 2b). In *V. chagasii*, genetic distances within the population were more homogenous,
173 being lower overall but larger than the within-clade distance in *V. crassostreae*.

174 Genomes of *V. chagasii* varied in size between 5.3 to 6.5 Mb (Table S1), which is indicative
175 of extensive flexibility of genome diversity among isolates. A total of 191 genes were
176 exclusively found in all *V. chagasii* isolates compared to only 53 specific genes in *V.*
177 *crassostreae* isolates (Table S2). Among the *V. chagasii* specific genes we found clusters
178 presumably involved in bacterial attachment (tight adherence and curling) and motility
179 (flagellar). On the other hand, none of the *V. chagasii* strains carried the virulence plasmid
180 pGV1512 that was present in 72% of *V. crassostreae* isolates and encodes a type 6 secretion
181 system cytotoxic for oyster immune cells (Piel, Bruto et al. 2020).

182 To analyze the diversity of phages isolated from *V. chagasii* we grouped them according to
183 their host, date of isolation and patterns of infectivity, resulting in 49 clusters for which we
184 investigated phage morphology, genome sequence, and taxonomy. Electron microscopy
185 revealed that all phages belong to the *Caudovirales*, including morphotypes of myoviruses
186 (n=15), podoviruses (n=22) and siphoviruses (n=11) (Fig.S2). Genome sequencing of these
187 double-stranded DNA viruses revealed extensive variability in genome length (from 31,648 to
188 161,442 bp) and gene content (45 to 305 predicted coding sequences) (Table S3). To explore
189 the genomic diversity of these phages we used the Virus Intergenomic Distance Calculator
190 (VIRIDIC, (Moraru, Varsani et al. 2020)) (Fig. 3a) which led us to group the 49 phages
191 infecting *V. chagasii* into 25 VIRIDIC genera (ANI>70%) and 41 species (ANI>95%). By
192 comparison, the higher number of 57 phages isolated using *V. crassostreae* were assigned to
193 only 19 genera and 23 species (Fig. 3b), indicative of a higher taxonomic diversity in our
194 collection of *V. chagasii* phages.

195 Only one genus (Genus 1, Podoviruses, Table S3) contained phages predicted as temperate
196 phages by BACPHILIP (score 0.975). A gene encoding a putative integrase (tyrosine
197 recombinase) was detected in the genomes of all 11 phages in this genus. However, this
198 prediction was contradicted by the absence of integration of the phages in *V. chagasii*
199 genomes. Tyrosine recombinase constitutes a large protein family involved in a wide variety
200 of biological processes (post-replicative segregation, genetic switches and movement of
201 mobile genetic elements including phages) (Smyshlyaev, Bateman et al. 2021) and functional
202 annotation of these proteins is still limited by their diversity and lack of experimental data.

203 Therefore, the classification of these phages as lysogens will have to be validated
204 experimentally.

205 We identified a single genus (Genus 10, Table S3) that contains phages infecting both *Vibrio*
206 species (Fig. 3a). This genus splits into two VIRIDIC species (identities >95%), the first
207 (species 38) containing a single phage isolated from *V. chagasii* and the second (species 15)
208 containing seven phages isolated from *V. crassostreae*. Among genes specific to VIRIDIC
209 species 15, we found a gene encoding a protein with a N-terminal phosphoadenosine
210 phosphosulphate reductase (PAPS) domain and a C-terminal DNA N-6-adenine-
211 methyltransferase (Dam) domain (Fig. S3). We previously demonstrated that this gene (called
212 PAPS-Dam) is involved in phage counter-defense against the DNA phosphorothioation-based
213 defense system Dnd (Piel, Bruto et al. 2022). We used Defense-Finder (Tesson, Hervé et al.
214 2021) to identify known defense systems in the hosts used to isolate the phages from Genus
215 10 and found the Dnd_ABCDEFGH system (Wang, Chen et al. 2011) in all *V. crassostreae*,
216 but not in *V. chagasii* hosts. This suggested that the PAPS-Dam phage anti-defense system is
217 involved in the adaptation of phages from species 15 to their *V. crassostreae* host.

218 Some genera of phages isolated from *V. crassostreae* or *V. chagasii* could be clustered in
219 VIRIDIC families (identities >50%). A core proteome phylogeny of the best represented
220 family indicated that phages differentiated from a common ancestor into genus and species in
221 their respective vibrio host species (Fig. 3a, Fig. S4). Neither *V. crassostreae*-infecting phages
222 nor *V. chagasii*-infecting phages form monophyletic clades, which indicates that phages have
223 jumped several times from one host species to another. To investigate evidence for past
224 recombination among phages, we compared the phylogenetic relationships of each core
225 protein (Fig. S5). Only 3 out of 38 proteins, a large terminase, a DNA binding protein and a
226 putative primase, cluster all *V. crassostreae*-infecting phages in a clade with 56, 100 and 85%
227 bootstrap values, respectively. Incongruences in other protein tree topologies suggest that
228 recombination has shuffled genes between phages. We speculate that vibrio diversity and
229 coinfection of oysters could enhance genomic diversification of the viral population,
230 promoting phage host jumps, as previously described for gut microbiota (De Sordi, Khanna et
231 al. 2017).

232 We analyzed host-phage interactions taking the vibrio core genome phylogeny and phage
233 clustering into consideration (Fig. 4a). In *V. chagasii*, the size of modules was significantly
234 smaller than in *V. crassostreae* (negative binomial GLM, estimate = 1.234 ± 0.241 , $z = 5.121$,

235 $p < 0.001$). Phages isolated using *V. chagasii* were more diverse than those from *V.*
236 *crassostreae*, which might at least partially explain the difference in module size. Smaller
237 modules might also indicate higher specialization of phages, i.e. a lower number of
238 susceptible hosts. Comparing pairwise number of shared phages and phylogenetic distance,
239 we found that the likelihood of sharing phages was highest at the smallest genetic distances
240 (Fig. 4b). Both species exhibited a multimodal distribution of sharing probabilities reflecting
241 the modular structure of the infection network. However, the distribution of these modes
242 differed substantially between the two species. While *V. chagasii* strains showed a
243 comparatively high sharing probability (~20%) at moderate genetic distances of 0.02, the
244 largest genetic distance with compatible phages was observed for *V. crassostreae* strains at
245 genetic distances larger than 0.04. This suggests a higher degree of specialization of *V.*
246 *chagasii* phages. In fact, only one phage (382E49-1) out of 49 was able to infect strains with
247 larger genetic distances (>0.024). We therefore conclude that smaller modules in *V. chagasii*
248 are a consequence of the lower number of genetically similar hosts, as well as a higher
249 diversity of the phages coupled to a higher degree of specialization.

250 With such a narrow host range, phages have to find strategies that increase encounter rates
251 with their host. On the one hand, *V. chagasii* inhabits an open system (seawater), which might
252 require a high viral load to promote phage-bacteria encounters. Individual strains of the most
253 abundant *Vibrio* species have previously been estimated to occur at concentrations of on
254 average 10^3 L^{-1} (Kauffman, Chang et al. 2022) and in the present study we showed that the
255 number of phages infecting a *V. chagasii* strain could reach $5 \cdot 10^3 \text{ L}^{-1}$ in seawater. On the other
256 hand, the oyster specialist *V. crassostreae* reaches a higher density in the animal host, which
257 might favor physical contact and promote phage infection. Furthermore, as the *V.*
258 *crassostreae* population is genetically structured by distinct clades of nearly clonal strains, the
259 same phage can potentially infect more hosts. Exploring to what extent clades bloom in
260 distinct oysters, co-occur in the same animal, or bloom sequentially, might be necessary to
261 decipher whether the loss of a specific *V. crassostreae* clade is frequently accompanied by the
262 rise of a population of phages which would be a clear indication that the latter moderate the
263 population of the former.

264 **Large numbers of phages during blooms generate epigenetic and genetic variability**

265 Blooms of specific strains can dramatically increase the abundances of specific phages as
266 observed in the time series on the 25th August (Fig. 1a, Fig. S1). The only large module

267 observed in the *V. chagasii* phage-bacteria network connecting hosts and phages was isolated
268 from samples taken on this date (Fig. 4a). On the host side, the phylogenetic relationship
269 based on the core genome revealed the grouping of eight strains in a clade with very little
270 intra-clade diversity (2 to 20 SNPs) (Fig. S6). Seven of these strains were isolated from
271 oysters sampled on the 25th August, and the absence of strain specific genes further confirmed
272 their clonality. The most closely related clade contains a group of four strains with higher
273 diversity (5 to 823 SNPs, 40 genes specific to strain 52_P_461 and 10 genes specific to strain
274 37_P_203) that were isolated from seawater collected before and after the 25th August.
275 Similarly, among the nine phages with sequenced genomes isolated from samples taken on
276 the 25th August, five belong to the same species (species 26, genus 18 Table S3) and differed
277 by a unique single nucleotide polymorphism (SNP) (Fig. S7). We compared the virulence of
278 these phages for all hosts from the two previously described sister clades using efficiency of
279 plating (EOP), i.e. the titer of the phage on a given bacteria compared to the titer on the strain
280 used to isolate the phage (original host) (Fig. 5a). This revealed that only phage 409E50-1
281 showed noticeable variation separating the four strains from the more diverse clade with low
282 susceptibility. These four low susceptibility (LS) strains showed significantly lower
283 susceptibility than the eight remaining clonal high susceptibility strains (HS, LM, estimate = -
284 3.322 ± 0.081 , $t = -40.609$, $p < 0.001$, Fig.5b). Lower infection could not be attributed to
285 difference in cell-surface phage receptors, as phage 409E50-1 adsorbed to all tested isolates
286 regardless of the production of progeny (Fig. S8). We thus concluded that an intracellular
287 defense system found in LS strains controls the efficiency of phage 409E50-1 infection, while
288 other phages from the same species are able to counteract this defense.

289 Genome comparisons revealed 276 genes that were present in all LS but absent in all HS
290 strains (Table S4). Among these we found two known phage defense systems, the
291 phosphorothioation-sensing bacterial defense system (sspBCDE) targeting viral DNA (Xiong,
292 Wu et al. 2020) and the cyclic-oligonucleotide-based anti-phage signalling systems
293 (CBASS_I) leading to host suicide (Millman, Melamed et al. 2020). However the double
294 deletion of these systems in 36_P_168 did not change the susceptibility of the host (not
295 shown). The defense mechanism(s) of LS acting on 409E50-1 therefore remain(s) to be
296 identified.

297 We also sought to understand how the other phages of this species escape the LS defense. A
298 phage can escape from host defenses by epigenetic or genetic modifications. Phages 409E50-
299 1 and 521E56-1 were isolated from different strains (respectively 50_O_409 and 56_P_521)

300 and diverge by a single SNP in their genome. To explore the hypothesis of epigenetic
301 modification, we produced these phages in the two host strains (Fig. 5c). When phage
302 409E50-1 was propagated on vibrio 56_P_521, its infectivity for LS strains was strongly
303 increased compared to the progeny obtained from the original host (LMM, estimate = $2.016 \pm$
304 0.296 , $t = 6.821$, $p = 0.002$). The infectivity of phage 521E56-1 was slightly increased when
305 produced on 50_O_409 compared to its original host (Fig. 5c, LMM estimate = $0.656 \pm$
306 0.062 , $t = 10.62$, $p < 0.001$). However, the effect of 409E50-1 was three times greater than
307 that of 521E56-1, indicating that the latter was less affected by the host used to produce
308 progenies. This suggests that vibrio strain 56_P_521 confers an epigenetic advantage to phage
309 409E50-1 and we hypothesize that phage 521E56-1 does not require host-mediated
310 modification because of genetic divergence.

311 Phage 409E50-1 differs from 521E56-1 by a single SNP localized in a gene encoding a
312 protein of unknown function (label VP409E501_p0076 in phage 409E50-1; 125 amino acid)
313 (Fig. S7). This SNP results in the change of a threonine (409E50-1) to a proline (521E56-1) at
314 amino acid position 8 (Fig. S9). We exchanged the allele T>G in phage 409E50-1 by
315 recombination (see Methods and Fig. 5d) and subsequently observed a significant decrease in
316 the infectivity of the control phages for LS strains (EOP of 10^{-6} instead of 10^{-4} for the
317 ancestral phage, Fig. 5b and 5c). Irrespective of this difference, control phage infections
318 showed significantly lower EOP on LS strains compared to the original host, 50_0_409 (LM,
319 estimate = -5.843 ± 0.123 , $t = -47.571$, $p < 0.001$). The recombinant phages (T>G) were on
320 average 10^6 fold more infectious than control phages on LS strains and did not differ
321 significantly from the original host, 50_0_409 (Fig. 5e, LM, estimate = -0.160 ± 0.120 , $t = -$
322 1.327 , $p = 0.196$). We therefore show that a single SNP provides protection from the
323 unknown phage defense system present in LS strains. We speculate that this locus could also
324 be the target for epigenetic protection conferred by the host 56_P_521 to phage 409E50-1.

325 With this mechanistic understanding of the bacteria-phage infection process, we can
326 hypothesize as to how defense elements could spread in the time series samples. The bloom-
327 like proliferation observed on the 25th August most likely resulted from the clonal expansion
328 of a HS strain. The expansion of a *V. chagasii* clone in oysters coincided with the
329 disappearance of *V. crassostreae* in the animals. This could be explained by biotic or abiotic
330 factors that are no longer conducive to the replication of *V. crassostreae* in the oyster, leaving
331 this niche accessible to the expansion of another population (population shift). Finally, the
332 clonal expansion presumably drove the overproportional increase of phages assigned to

333 species 26. It is conceivable that this large phage population generated genetic diversity by
334 mutation, including the previously identified SNP that led to adaptation to the host defense
335 system. This adaptation enabled the predominant phages to reproduce in LS strains after the
336 HS bloom and persist for prolonged periods also outside of the oyster environment.

337 **CONCLUSION**

338 In this study we explored how different genetic structures of hosts can feed back on host-
339 phage interaction networks and genetic diversity of both hosts and phages. The different
340 genetic structures also reflect differences in host ecology and may therefore also influence
341 phage ecology (host range and population dynamics). Between host species, contrasting
342 patterns of genetic diversity for both host and phage resulted in different structures of the
343 respective phage-bacteria interaction networks. Smaller modules in *V. chagasii* were a
344 consequence of a lower number of clonal hosts. More diverse hosts used as prey will lead to
345 the isolation of more diverse phages. A higher specialization of the phages infecting *V.*
346 *chagasii* was, however, supported by the highest likelihood to share phages when genetic
347 distances were small. Host blooms result in a high number of phages and we showed that this
348 can generate adaptive genetic variability. Selection can work on this increased variability and
349 may favor variants that can also infect hosts outside the bloom, that were less susceptible to
350 the original phage. The free living lifestyle of *V. chagasii* with blooms inside and outside
351 oyster hosts may thus increase genetic variability of phages, but prevent the formation of
352 modules in this host with low population structure.

353 **MATERIAL AND METHODS**

354 **Sampling**

355 Sampling was performed at the same time as for Piel et al. (2022). Briefly, samples
356 were collected from an oyster farm located in the Bay of Brest (Pointe du Château, 48° 20'
357 06.19" N, 4° 19' 06.37" W) every Monday, Wednesday and Friday from the 3rd May to the
358 11th September 2017. Specific Pathogen Free (SPF) juvenile oysters (Petton, Bruto et al.
359 2015) were deployed in the field in batches of 100 animals. When the first mortalities were
360 observed in the first batch, another batch of SPF animals was placed in the field, leading to
361 the consecutive deployment of seven batches from the 26th April to the 11th September.
362 Oyster mortalities were recorded on each sampling day. Seawater temperature reached 16°C
363 on the 22nd May, a previously observed threshold for oyster mortalities (de Lorgeril, Lucasson

364 et al. 2018). Mortalities began on the 29th May and persisted until the 25th August. On each
365 sampling date, five living oysters were collected from a batch showing less than 50%
366 mortality. The animals were cleaned, shucked, weighed and 2216 Marine Broth (MB) was
367 added (10 mg/ml) for homogenization using an ultra-turrax. 100 µL of the homogenate was
368 used for vibrio isolation, with the remaining volume centrifuged (10 min, 17,000 g) and the
369 supernatant filtered through a 0.2 µm filter and stored at 4°C until the phage isolation stage.
370 Two liters of seawater were collected and size fractionated as previously described (Bruto,
371 James et al. 2017). Bacterial cells from 0.2 µm filters were suspended in 2 mL MB and 100
372 µL of this suspension was used for vibrio isolation. The iron chloride flocculation method was
373 used to generate 1000-fold concentrated viral samples from 2 liters passed through a 0.2 µm
374 filter, following the previously described protocol (Kauffman, Brown et al. 2018). Virus-
375 flocculates were suspended in 2mL 0.1M EDTA, 0.2M MgCl₂, 0.2M oxalate buffer at pH6
376 and stored at 4°C until the phage isolation stage.

377 ***Vibrio* isolation, identification and genome analysis**

378 *Isolation and identification.* Vibrios from seawater or oyster tissues were selected on
379 thiosulfate-citrate-bile salts-sucrose agar (TCBS). Roughly 48 colonies were randomly picked
380 from each plate and re-isolated once on TCBS, then on 2216 Marine agar (MA). Colonies
381 were screened by PCR targeting the *r5.2* gene encoding for a regulator (Lemire, Goudenege et
382 al. 2014). PCR positive isolates were grown in MB and stored at -80°C in 10% DMSO.
383 Bacteria were grown overnight in 5mL MB and DNA extracted using an extraction kit
384 (Wizard, Promega) according to the manufacturer's instructions. Taxonomic assignment was
385 further refined by *gyrB* gene sequencing to identify *V. crassostreae* and *V. chagasii* isolates.
386 The partial *gyrB* gene was amplified using degenerate primers (Table S5), Sanger sequenced
387 (Macrogen), with sequences manually corrected by visualization of chromatograms.
388 Sequences were aligned with Muscle and phylogenetic reconstruction was performed with
389 RAxML version 8 GTR model of evolution, a gamma model and default parameters
390 (Stamatakis 2006).

391 *Genome sequencing, assembly and annotation.* *V. chagasii* libraries were prepared from
392 500 ng of genomic DNA using MGIEasy Universal DNA Library Prep Set following the
393 manufacturer's instruction. The 100bp paired-end library pools were circularised and
394 sequenced using DNBSEQ-G400 (BGI) by the Biomix platform at the Pasteur Institute
395 (Paris, France). Reads were trimmed using Trimmomatic v0.39 (LEADING:3, TRAILING:3,

396 SLIDINGWINDOW:4:15, MINLEN:36) (Bolger, Lohse et al. 2014). *De novo* read assembly
397 was performed using Spades version 3.15.2 (--careful --cov-cutoff auto -k 21,33,55,77 -m 10).
398 In order to confirm BGI sequencing quality, we compared these assembly results with those
399 obtained for 20 of the same *V. chagasii* strains sequenced by Illumina HiSeq using QUA
400 ST v5.0.2 (Gurevich, Saveliev et al. 2013) (Table S6). We confirmed the previous benchmark
401 studies showing similar sequencing and assembly qualities across both platforms (Foux,
402 Tighe et al. 2021). Computational prediction of coding sequences and functional assignments
403 were performed using the automated annotation pipeline implemented in the MicroScope
404 platform (Vallenet, Belda et al. 2013). Phage defense gene annotation was performed using
405 Defense-Finder version 1.0.8 (Tesson, Herve et al. 2022) with MacSyFinder models version
406 1.1.0 and default options. Persistent genome phylogeny was undertaken using the PanACoTA
407 workflow (Perrin and Rocha 2021) version 1.3.1-dev2 with mash (version 2.3) distance
408 filtering from 0 to 0.3, prodigal version 2.6.3 for syntactic annotation, mmseqs version
409 2.11.e1a1c for gene clustering, mafft version 7.407 for persistent genome alignment defined
410 as 90.0% of all genomes in each family and IQ-TREE (Nguyen, Schmidt et al. 2015) version
411 2.0.3 based on GTR model and 1000 bootstrap for tree construction.

412 *Comparative genomics.* The average nucleotide identity (ANI) value of genomes was
413 determined using fastANI version 1.32 (fragment length 500) (Jain, Rodriguez et al. 2018). *V.*
414 *crassostreae* and *V. chagasii* core, variable, accessory and specific genomes were established
415 using mmseqs2 reciprocal best hit version 13-45111 with 80% identities on 80% coverage
416 thresholds (Steinegger and Soding 2018). Synteny plots were generated using dedicated
417 python scripts based on the “DNA Features Viewer” library ([https://github.com/Edinburgh-](https://github.com/Edinburgh-Genome-Foundry/DnaFeaturesViewer)
418 [Genome-Foundry/DnaFeaturesViewer](https://github.com/Edinburgh-Genome-Foundry/DnaFeaturesViewer)) and clinker for conservation representation
419 (<https://github.com/gamcil/clinker>).

420 **Phage isolation, identification and genome analysis**

421 *Phage isolation.* We used the 194 and 252 isolates of *V. crassostreae* and *V. chagasii*
422 respectively as ‘bait’ to isolate phages from the same date of sampling from 20mL-seawater
423 equivalents of viral concentrate (1,000X) or oyster tissues (0.1mg). Phage infection was
424 assessed by plaque formation in soft agar overlays of host lawns mixed with both viral
425 sources. For *V. crassostreae*, due to the limited number of co-occurring phages, the collection
426 was completed by isolating viruses from seawater concentrates sampled on several dates
427 (Piel, Bruto et al. 2022). For *V. chagasii*, we purified one phage from each plaque positive

428 host, representing a final set of 95 phage isolates. To generate high titer stocks, plaque plugs
429 were first eluted in 500 µl of MB for 24 hours at 4°C, 0.2-µm filtered to remove bacteria, and
430 re-isolated three times on the sensitive host for purification before storage at 4°C and, after
431 addition of 25% glycerol, at -80°C. High titer stocks ($>10^9$ PFU/ml) were generated by
432 confluent lysis in agar overlays and phage concentration was determined by dropping spots of
433 ten-fold serial dilutions onto bacterial host lawns.

434 *Electron microscopy.* Following concentration on centrifugal filtration devices
435 (Millipore, amicon Ultra centrifugal filter, Ultracel 30K, UFC903024), 20 µl of the phage
436 concentrate were adsorbed for 10 min to a formvar film on a carbon-coated 300 mesh copper
437 grid (FF-300 Cu formvar square mesh Cu, delta microscopy). The adsorbed samples were
438 negatively contrasted with 2% uranyl acetate (EMS, Hatfield, PA, USA). Imaging was
439 performed using a Jeol JEM-1400 Transmission Electron Microscope equipped with an
440 Orious Gatan camera at the MERIMAGE platform (Station Biologique, Roscoff, France).

441 *DNA extraction.* Phage suspensions (10 mL, $>10^{10}$ PFU/mL) were concentrated to
442 approximately 500µL on centrifugal filtration devices (30 kDa Millipore Ultra Centrifugal
443 Filter, Ultracel UFC903024) and washed with 1/100 MB to decrease salt concentration. The
444 concentrated phages were next treated for 30 min at 37°C with 10 µL of DNase (Promega)
445 and 2.5µL of RNase (Macherey-Nagel) at 1000 units and 3.5 mg/mL, respectively. Nucleases
446 were inactivated by adding EDTA (20 mM, pH8). DNA extractions involved a first protein
447 lysis step (0.02 M EDTA pH 8.0, 0.5 mg/mL proteinase K, 0.5% SDS) for 30 min incubation
448 at 55°C, followed by phenol chloroform extraction and ethanol precipitation. DNA was
449 visualized by agarose gel electrophoresis (0.7% agarose, 50V, overnight at 4°C) and
450 quantified using QuBit.

451 *Genome sequencing, assembly.* Phages were sequenced by the Biomics platform at the
452 Pasteur Institute (Paris, France). The sequencing library (2 × 75 bp paired end) was prepared
453 using a TruSeq Illumina kit and sequenced on a NextSeq550 Illumina sequencer. After read
454 trimming conducted with Trimmomatic v0.39 (LEADING:3, TRAILING:3,
455 SLIDINGWINDOW:4:15, MINLEN:36) (Bolger, Lohse et al. 2014), de novo assembly was
456 performed using SPAdes v3.15.2 (--careful --cov-cutoff auto -k 21,33,55,77 -m 10). Vector
457 contamination contigs were excluded using the UniVec Database and the one-contig phage
458 genome was manually decircularized. Syntactic annotation was performed with
459 PHANOTATE v1.5.0 (McNair, Zhou et al. 2019). Large terminase subunit was identified

460 using DIAMOND v2.0.8.146 (blastp, default parameters) (Buchfink, Reuter et al. 2021) on
461 previously annotated vibrio large terminase and using HMMSCAN (HMMER v3.3.2, default
462 parameters with $\text{evalue} \leq 10^{-3}$) with PFAM profiles (PF03354.17, PF04466.15, PF05876.14,
463 PF06056.14) (Mistry, Chuguransky et al. 2021). The phage genome was manually ordered
464 starting from the gene for the large terminase subunit.

465 *Genome annotation.* The tRNA were identified with tRNAscan-SE v2.0.9 (Chan and
466 Lowe 2019). Functional annotation used multiple approaches. First, we used HMMSCAN
467 ($\text{evalue} \leq 10^{-3}$) with PVOGS profiles (Grazziotin, Koonin et al. 2017). We also used
468 DIAMOND blastP against first the Nahant collection genomes and then against other
469 bacterial RefSeq (version 212) and phage GenBank genomes (2022-24-06, with 30%
470 identities and 50% coverage) (Kauffman, Brown et al. 2018, Buchfink, Reuter et al. 2021).
471 Finally, we added InterProScan v5.52-86.0 ($\text{evalue} \leq 10^{-3}$) and eggNOG v5.0.2 annotation
472 results (Jones, Binns et al. 2014, Huerta-Cepas, Szklarczyk et al. 2019).

473 *Clustering, lifestyle and comparative genomics.* We clustered phages using VIRIDIC
474 v1.0r3.6 (default parameters) (Moraru, Varsani et al. 2020). Intergenomic similarities were
475 identified using BLASTN pairwise comparisons. Virus assignment into family ($\geq 50\%$
476 similarities), genera ($\geq 70\%$ similarities) and species ($\geq 95\%$ similarities) ranks followed the
477 International Committee on Taxonomy of Viruses (ICTV) genome identity thresholds. Phage
478 lifestyle was predicted using BACPHILIP version 0.9.3-alpha and lysogeny associated genes
479 were searched using InterProScan (v.5.52-86.0) based on integrase (IPR002104), resolvase
480 (IPR006119), replicative transposases (IPR004189), and transcriptional repressor domains
481 (IPR010982, IPR010744, IPR001387, IPR032499). Absence of prophage integration was also
482 checked using BLASTn searches between both phage and bacterial genomes ($\text{evalue} \leq 0.01$)
483 (Hussain, Dubert et al. 2021). All comparative genomic analyses were conducted using
484 MMseqs2 version 13-45111 for protein clustering (Steinegger and Soding 2018), FAMSA
485 version 1.6.2 for gene/protein alignments (Deorowicz, Debudaj-Grabysz et al. 2016) and IQ-
486 TREE version 2.1.2 for phylogenetic tree construction (Nguyen, Schmidt et al. 2015).

487 **Host range determination**

488 *Single-phage-by-single-host host range infection assay.* Host range assays were carried
489 out using an electronic multichannel pipette by spotting 5 μL of the phage suspension
490 normalized at 2×10^5 PFU/ml (10^3 PFU/spot) on the agar overlay inoculated with the tested

491 host. Plates were incubated overnight at RT and plaque formation was observed after 24
492 hours. Spot assays were performed for *V. chagasii*, first by infecting 358 vibrio strains (252 *V.*
493 *chagasii* + 106 strains belonging to other vibrio populations) with 95 phages in duplicate.
494 Then, the host range of 49 selected phages was confirmed on 136 *V. chagasii* hosts.
495 Concerning *V. crassostreae*, results were obtained in (Piel, Bruto et al. 2022).

496 *Efficiency of plating (EOP).* Ten-fold serial dilutions of phages from a high titer stock
497 ($>10^9$ PFU/mL) were prepared and 10 μ L of each dilution were pipetted onto bacterial host
498 lawns. The EOP was calculated as the ratio between the titer of a phage on a given strain
499 compared to the titer of the same phage on its reference strain (host used to isolate and
500 produce the phage).

501 *Adsorption estimation.* Phage adsorption experiments were performed as previously
502 described (Hyman and Abedon 2009). Phages were mixed with exponentially growing cells
503 (OD 0.3; 10^7 CFU/mL) at a MOI of 0.01 and incubated at RT without agitation. At different
504 time points, 250 μ L of the culture was transferred into a 1.5 mL tube containing 50 μ L of
505 chloroform and centrifuged at 17,000g for 5 min. The supernatant was 10-fold serially diluted
506 and drop spotted onto a fresh lawn of a sensitive host to quantify the remaining free phage
507 particles. In this assay, a drop in the number of infectious particles at 15 or 30 min indicates
508 bacteriophage adsorption.

509 **Statistical analyses**

510 Statistical analyses were based on linear models (LM) when data were normally
511 distributed or on negative binomial generalized linear models (negbin GLM) when data were
512 left skewed. Correlations were based on non-parametric Spearman's rank correlations. Paired
513 tests were analysed as linear mixed models adding the paired factor as a random intercept. All
514 analyses were performed using the R statistical environment (v.4.0.5).

515 **Molecular microbiology**

516 *Strains and plasmids.* All plasmids and strains used or constructed in the present study
517 are described in Table S7 and S8. *V. chagasii* isolates were grown in Marine broth (MA) or
518 LB+0.5 M NaCl at RT. *Escherichia coli* strains were grown in LB at 37°C. Chloramphenicol
519 (Cm; 5 or 25 μ g/mL for *V. chagasii* and *E. coli*, respectively), thymidine (0.3 mM) and
520 diaminopimelate (0.3 mM) were added as supplements when necessary. The P_{BAD} promoter

521 was induced by the addition of 0.2% L-arabinose to the growth media and repressed by the
522 addition of 1% D-glucose. Conjugation between *E. coli* and *V. chagasii* were performed at
523 30°C as described previously (Le Roux, Binesse et al. 2007).

524 *Cloning.* For vibrio knock outs, all cloning in the suicide vector pSW7848T was
525 performed using Herculase II fusion DNA polymerase (Agilent) for PCR amplification and
526 the Gibson Assembly Master Mix (New England Biolabs, NEB) according to the
527 manufacturer's instructions. For recombination of the 409E50-1 phage, a 227 bp region
528 flanking the SNP (T in 409E50-1, G in other phages from species 26) was amplified using the
529 Herculase II and DNA from the 521E56-1 phage and cloned by Gibson Assembly in pMRB
530 (Le Roux, Davis et al. 2011) instead of the *gfp* gene. All cloning was firstly confirmed by
531 digesting plasmid minipreps with specific restriction enzymes and secondly by sequencing the
532 insert (Eurofins).

533 *Vibrio mutagenesis.* Knock out was performed by cloning 500bp fragments flanking the
534 pSW7848T region (Le Roux, Binesse et al. 2007). This suicide vector encodes the *ccdB* toxin
535 gene under the control of an arabinose-inducible and glucose-repressible promoter, *P_{BAD}*.
536 Selection of the plasmid-borne drug marker on Cm and glucose resulted from integration of
537 pSW7848T in the genome. The second recombination leading to pSW7848T elimination was
538 selected on arabinose-containing media.

539 *Phage mutagenesis.* Recombinant phage (T>G in p0076 of phage 409E50-1) was
540 engineered using double crossing over with a plasmid carrying a 227 region of homology to
541 the phage genome 521E56-1 (Fig. 5d). This plasmid, or as control the original pMRB-*gfp*,
542 was transferred by conjugation to strain 50_O_409. Plate lysates were generated by mixing
543 100 µL of an overnight culture of the transconjugant with the 409E50-1 phage and plating in
544 5 mL agar overlay. After the development of a confluent lysis of lawns, the lysate was
545 harvested by addition of 7 mL of MB and shredding of the agar overlay and stored ON at 4°C
546 for diffusion of phage particles. The lysates were next centrifuged, the supernatant filtered
547 through 0.2 µm filter and stored at 4°C. We could not enrich the recombinant phages by
548 infecting LS strains because these strains confer epigenetic protection to the 409E50-1 phage.
549 We therefore performed several successive passages of the phage on the strain carrying the
550 plasmid, testing the infectivity of the phages on the original strain and the LS strain at each
551 passage. After three passages, a different titer between the putative recombinant and controls
552 was observed. The p0076 gene was PCR amplified using single plaque as template (eight

553 recombinant or eight control phages) and primers flanking the 227 region in the phage
554 genome (external primers). The SNP was confirmed by sequencing. Three control or
555 recombinant phages were tested by EOP on four LS strains in addition to the source
556 50_O_409.

557 **Data availability**

558 Sequenced genomes have been deposited under the ENA Project with accession numbers
559 PRJEB53320 for *V. chagasii* and PRJEB53960 for phages.

560 **Materials availability**

561 All vibrio strains, phage strains and plasmids are available upon request.

562

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576 **Competing interests**

577 Authors declare no competing interests.

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750

751 **TITLES AND LEGENDS TO FIGURES**

752

753 **Figure 1. Time-series sampling of *V. chagasii* and vibriophages**

754 **a**, On each sampling date, vibrios from seawater (1-0.2 μ m size fraction) or tissue from five
755 oysters were selected on TCBS and genotyped to identify *V. chagasii* isolates. Oyster
756 mortalities occurred between May 29th-August 25th (black bar) and *V. crassostreae* was
757 detected in oysters from June 16th-August 18th (pink bar). Green lines show the frequency of
758 *V. chagasii* (number of positive isolates out of 48 randomly picked colonies*100) in seawater
759 (solid) or oyster tissue (dashed). Each of the *V. chagasii* isolates was used as "bait" to detect
760 co-occurring lytic phages by plaque formation in soft agar overlays of host lawns mixed with
761 viral concentrate from 1) seawater and 2) oyster tissues. Black circles and triangles indicate
762 the mean number \pm se of plaque forming units (PFU) obtained for each plaque positive host
763 using viruses collected from seawater (circles) or oyster tissue (triangles) collected on the
764 same day. The red star indicates the date of the *V. chagasii* bloom (25th August). **b**,
765 Correlation between mean number of PFUs and *V. chagasii* frequency for viral concentrates
766 from seawater (upper panel) or oysters (lower panel). Regression lines show similar
767 correlation estimates for both isolation fractions (seawater: estimate = 0.627 ± 0.193 , $t =$
768 3.252 , $p = 0.004$; oyster: estimate = 0.849 ± 0.342 , $t = 2.487$, $p = 0.022$).

769

770 **Figure 2. Genome diversity of *V. crassostreae*. and *V. chagasii*.** **a**, Core genome phylogeny
771 based on 2689 gene families of 88 *V. crassostreae* and 136 *V. chagasii* isolates from the time
772 series, with pairwise ANI values revealing highly structured clades in *V. crassostreae*, but not
773 in *V. chagasii*. Clade numbers refer to (Piel, Bruto et al. 2022). **b**, The resulting distribution of
774 pairwise genetic distance within *V. chagasii* (green) and *V. crassostreae* (pink) indicates
775 striking differences between these species (Two-sample Kolmogorov-Smirnov test, $D =$
776 0.762 , $p < 0.001$): *V. chagasii* exhibits a unimodal distribution with few clonal strains and a
777 homogenous pairwise genetic distances with a maximum differentiation of 0.024, whereas *V.*
778 *crassostreae* exhibits a multimodal distribution corresponding to the distinct clades with low
779 within-clade and large between clade genetic distances.

780

781 **Figure 3. Diversity of phages isolated using *V. chagasii* and *V. crassostreae* as hosts.** **a**,
782 VIRIDIC intergenomic similarity between phage genomes. The 106 phages included in the
783 study were grouped into 43 clusters assigned to VIRIDIC genus rank (>70% identities,
784 indicated with a plain line). Only one genus contains phages isolated using *V. crassostreae* or

785 *V. chagasii*. The phylogenetic tree based on 41 core proteins (25% identities and 80% coverage)
786 shows that this genus splits into two clades, one represented by a single phage isolated from
787 *V. chagasii* and the second containing seven phages all isolated from *V. crassostreae*. Some
788 genera were grouped into clusters assigned to VIRIDIC family rank (>50%). For the larger
789 family, the phylogenetic tree based on 38 core proteins (25% identities and 80% coverage)
790 delineates the phages isolated from *V. crassostreae* and *V. chagasii* populations.
791 **b**, The 49 and 57 phages infecting *V. chagasii* and *V. crassostreae* were assigned to 25 and 18
792 genera and 41 and 23 species, respectively.

793

794 **Figure 4. Modularity is driven by phylogenetic distances within the host population. a**,
795 Comparisons of the phage-bacteria infection matrix for *V. crassostreae* and *V. chagasii* as
796 hosts. Rows represent sequenced *Vibrio* strains ordered according to the Maximum
797 Likelihood core genome phylogeny of *V. chagasii* (n=136 green matrix) and *V. crassostreae*
798 strains (n=88; pink matrix). Columns represent sequenced phages (49 and 57 isolated from *V.*
799 *chagasii* and *V. crassostreae*) ordered by VIRIDIC genus (see dendrogram in Fig. 3a).
800 Colored squares indicate killing of the respective vibrio isolate by the phage. Vertical lines
801 represent VIRIDIC genus (solid) and species (dashed). **b**, Sharing probability (i.e. proportion
802 of phages shared in all pairs within a window of genetic distances) weighed by the frequency
803 of pairwise comparisons (i.e. number of pairwise comparisons within each window divided by
804 the total number of pairwise comparisons) as a function of host genetic distance. Sharing
805 probability was assessed in sliding windows with an interval size of 0.003 and a step size of
806 0.001 along observed genetic distances.

807

808 **Figure 5: Epigenetic and genetic modification are involved in phage adaptation. a**,
809 Efficiency of plating (EOP, the titer of the phage on a given bacteria divided by the titer on
810 the strain used to isolate the phage or 'original host') of six phages from VIRIDIC species 26
811 (Fig. S6) on 12 *V. chagasii* strains. Individual dots correspond to the mean from three
812 independent experiments. **b**, EOP of the phage 409E50-1 infecting *V. chagasii* strains with
813 low susceptibility (LS) or high susceptibility (HS). Bar charts show the mean +/- s.d. from
814 three independent experiments (individual dots). **c**, EOP obtained using *V. chagasii* strains
815 50_O_409 (HS) or 56_P_521 (LS) to produce the phages 409E50-1 or 521E56-1. Results
816 were standardized by experiment, i.e. the production and testing on the original host was
817 normalized to 1 for each experiment. **d**, Graphic summary of the strategy used to exchange
818 the nucleotide T to G and codon Thr to Pro in phage 409E50-1. **e**, EOP of the recombinant

819 phages or control infecting the four *V. chagasii* strains with low susceptibility (LS). Results
820 were standardized by experiment, i.e. the production in original host (50_0_409) was
821 normalized to 1 for each experiment.