Modelling bacteria-phage interactions driving predation and horizontal gene transfer

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ABSTRACT

Bacteriophages shape microbial communities by predating on them and by accelerating their adaptation through horizontal gene transfer. The former is the basis of phage therapy, whereas the latter drives the evolution of numerous bacterial pathogens. We present a novel computational approach (eVIVALDI – eco-**eV**olutionary m**I**crobial indi**V**idu**AL**-base**D** s**I**mulations) to study bacteria-phage ecological interactions that integrates a large number of processes, including population dynamics, environmental structure, genome evolution, and phage-mediated horizontal transfer. We validate and illustrate the relevance of the model by focusing on three specific questions: the ecological interactions between bacteria and virulent phage in the context of phage and antibiotic therapy, the role of prophages as competitive weapons, and autotransduction leading to bacterial acquisition of antibiotic resistance genes upon lysis of resistant competitors. Our model recapitulates experimental and theoretical observations and provides novel insights. In particular, we find that environmental structure has a strong effect on community dynamics and evolutionary outcomes in all three case studies. Strong environmental structure, especially if antibiotics are heterogeneously distributed, promotes the acquisition of resistance to both phages and antibiotics, creates variation in the dynamics of arm-races between bacteria and phage, and better predicts dynamics of lysogen invasion in the gastrointestinal tract, compared to models assuming well-mixed environments. Moreover, we predict a parameter space where co-existence between invaders and resident lysogens can occur during autotransduction, which we then confirm experimentally. By linking ecological and evolutionary dynamics, our modelling approach sheds light on the factors that influence the dynamics of bacteria-phage interactions. It can also be expanded to put forward novel hypotheses, facilitating the design of phage therapy treatments and the assessment of the role of phages in the spread of antibiotic resistance.

Introduction

Microbial organisms are pervasive across all natural environments, including the human body. Their adaptation and organization in communities may lead to disease [1], drive host evolution [2], and produce major changes in ecosystems [3,4]. Ecological interactions in microbial communities influence, and are influenced by, the rapid pace with which microbes acquire adaptive changes [5,6]. A striking example is the relationship between bacteria and bacteriophages (from here on referred to as phages), because the latter predate on the former whilst also driving their adaptation [4]. Phages are the most abundant entities in nature [7,8] and very efficient bacterial predators; it has been estimated that they promote the turnover of ~20% of bacterial mass every single day in certain environments [9,10]. In the context of widespread antibiotic resistance, this has led to a rekindled interest in phage therapy as an adjuvant or a replacement of antibiotic therapy against multi-resistant bacteria [11].

Virulent phages follow a strictly lytic cycle within their hosts, but they often exist in diverse communities with other virulent and temperate phages. Infection by the latter can lead to either the lytic cycle or their integration in bacterial genomes, as prophages (lysogenic cycle). Temperate phages are not used for phage therapy because lysogeny prevents them from extinguishing bacterial populations and drives the latter's resistance to closely, and sometimes distantly related phages – a mechanisms called superinfection exclusion [12-14]. However, half of the bacterial genomes contain at least one, and up to 20, prophages, and these are more frequent in bacterial pathogens [15], which means that they cannot be ignored in phage therapy. The expression of prophage genes may provide novel phenotypes to the host (lysogenic conversion), and many cases have been described where prophages carry adaptive traits implicated in virulence or resistance to stress [16]. Virions arising from prophage induction can infect closely related competitor bacteria that are non-lysogenic for the phage, decreasing bacterial competition,

61 increasing prophage frequency, and liberating resources that can be used for the growth of the
62 remaining lysogenic population [17]. In this case, prophages have been regarded as weapons
63 against bacterial competitors [13,18].

64 Phages can drive horizontal gene transfer between bacteria by transduction [19]. This can be a
65 hazard in the case of phage therapy if the transferred traits are virulence factors or antibiotic
66 resistance genes. Specialized transduction occurs in temperate phages when erroneous prophage
67 excision leads to the transfer of neighboring chromosomal genes. Generalized transduction
68 occurs when bacterial DNA is delivered to other cells after being mistakenly encapsulated in
69 virions, due to the specificities of the *pac* DNA packaging system [20]. Although these mechanisms
70 are commonly used as genetic engineering tools [21], their rates in nature are poorly known. In
71 the lab, they vary across several orders of magnitude (between 10^{-11} and 10^{-3} [22,23]), depending
72 on the phage, the environment, and the type of culture media [19]. Importantly, phage driven
73 transmission of bacterial DNA can have particularly nefarious consequences for humans.
74 Transducing phages are responsible for the transmission of virulence factors in *Staphylococcus*
75 *aureus* [24], and may accelerate the spread of antibiotic resistance genes [25,26]. Transduction
76 can also have an impact at very short time scales: prophage induction facilitates the capture of
77 adaptive traits (e.g., an antibiotic resistance gene) from a second bacterial strain that is infected
78 by the phage and, through generalized transduction, transfers the gene to the lysogenic strain.
79 This process has been called autotransduction [27]. Hence, phages drive the evolution of bacterial
80 gene repertoires and may spread virulence or antibiotic resistance factors during phage therapy.

81 The diversity of interactions between phages and bacteria may obscure the effects of each of
82 them. Experimental approaches have described and clarified the mechanisms underlying these

83 interactions, but usually focused on simplified environments [8,28]. *In vivo* studies of these
84 interactions (e.g., in mammalian hosts [29]) tackle more natural environments, but have limited
85 resolution in tracking temporal dynamics or the effects of individual mechanisms. Mathematical
86 modelling provides a complementary approach to the study of phage-bacteria interactions,
87 providing important insights on their co-evolutionary processes [30] or the dynamics of particular
88 bacterial defense mechanisms [31,32]. Previous models have focused on individual mechanisms
89 of interaction in simple environments (*e.g.*, how the evolution of resistance to phage can affect
90 clinical treatments [33]), because tackling multiple mechanisms and spatial heterogeneity hinders
91 the development of analytical solutions. Yet, natural communities, and particularly those relevant
92 for phage therapy, include complex interactions and spatial structure [34-37]. This may explain
93 why models sometimes fail to fully reproduce *in vivo* dynamics of phage infection [28], and why
94 there is paucity of models on the impact of phage-mediated horizontal gene transfer in the
95 adaptation of bacterial communities (but see the work of Volkova et al [38] for a theoretical
96 comparison between the relative efficacy of transduction versus conjugation in transmitting an
97 adaptive trait).

98 Individual-based models (IBMs) are an alternative to population-based mathematical approaches
99 for studying complex microbial systems [39], and they have been useful to understand, for
100 instance, the interactions between bacteria and phages in biofilms [36]. Although computationally
101 intensive, IBMs provide a framework to study biological systems through the incorporation of
102 different (and potentially interacting) mechanisms at the level of the individual. Population-level
103 dynamics can then emerge from the collective individual behaviors. This makes IBMs particularly
104 appealing to investigate bacteria-phage interactions, because these involve both ecological (e.g.,
105 predation) and evolutionary (e.g., transduction of adaptive traits) scales, with antagonistic

mechanisms defined at the individual level (e.g., the lysis-lysogeny decision of temperate phage). To study these multiple roles of phages in microbial communities, we developed an IBM approach that is able to simulate diverse mechanisms and eco-evolutionary contexts: eVIVALDI – eco-**eV**olutionary m**I**crobial indi**V**idu**AL**-base**D** s**I**mulations. We focus on three questions, of gradually increasing complexity, that are relevant for bacterial evolution and phage therapy, and that cover a good range of possible eco-evolutionary interactions between bacteria and phage. First, we introduce the basic scheme of the simulation with the study of ecological interactions between co-evolving virulent phages and bacteria under phage and antibiotic pressure in structured environments. Then we introduce lysogeny and super-infection exclusion in the model to study the role of prophages as competitive weapons. We show that our model provides better fit to previous experimental results than earlier models. Finally, we introduce transduction and the way we encode individual genomes in the model to elucidate how bacteria may obtain novel adaptive genes from sensitive bacteria by autotransduction. We use eVIVALDI to explore and quantify the different mechanisms of phage-bacteria interactions and to gain insights on how the structure of the environment can affect these interactions and the community dynamics. We tackle each question by demonstrating the ability of the model to capture previous results and then show how its complexity highlights new relevant features.

Methods

Concept and basic implementation

The eVIVALDI model was developed in Python (version 2.7.3), using an object-oriented approach, with a focus on the flexibility and extensibility of mechanisms and parameters simulated. The complete ODD (Overview, Design concepts, and Details) protocol [40] of the developed model is available as supplementary text (Text S1), but below is a brief overview of the model. The source of the software can be obtained from the authors. The simulations can be run on a typical desktop computer. In a 3GHz 8-core Mac Pro, with 32GB of RAM, a replicate of a simulation (100 iterations), takes from ~5 to 30 minutes, depending on the parameters. Computations can also be performed in a cluster, allowing the parallel simulation of multiple parameters.

Entities and their ecological setup

Both bacterial cells and phage particles are represented as independent individuals on an environment represented as a two-dimensional grid with Moore neighbourhood (the 8 connected grid spaces of each location, for a Moore distance of one) (Fig 1A). Bacteria can be of different species. Each individual bacterium has a genome with core, accessory and, eventually, prophage genes. Bacteria have individual phenotypes, such as growth rate or the ability to survive antibiotic exposure. Phages can be from different species, have different lifestyles (temperate, virulent or defective), and possess individual phenotypes (e.g., attachment receptors and burst sizes). The host range of phage hosts is defined by a matrix (Fig 1B), and the superinfection exclusion rules amongst phages is defined in a similar way (Fig 1C).

Environmental and bacterial updates

The environment and the individuals are updated and behave according to biologically inspired rules. The environment can be completely structured, semi-structured or not structured at all (i.e.,

146 well-mixed), and it can be set as bounded or have a toroidal space. The type of structure influences
147 the diffusion of the different bacterial cells and environmental particles (phage and antibiotics).
148 Each location can hold a single bacterial cell and several phage cells. Free space is the bacterial
149 resource to be consumed, and it is freed whenever bacteria die. Bacterial death can be
150 intrinsic (e.g., of old age) or explicit (e.g., exposure to antibiotics or predation by phage) (Fig 1D).
151 When a free space is available, the neighboring bacteria compete for reproduction. The outcome
152 of the competition is chosen through a roulette wheel method that accounts for the fitness of each
153 bacterium. The successful bacterium generates an offspring into the free space (Fig 1E). Bacteria
154 can be infected by phage in the environment. The outcome of the infection depends on the phage
155 lifestyle and, for temperate phage, the lysis-lysogeny decision. This decision is stochastic but
156 influenced by the number of surrounding phages. For temperate phage, integration in the host
157 genome means vertical inheritance with host replication, until the phage excises from the genome,
158 according to a probability that can be low but non-null throughout the simulation (stochastic
159 prophage induction) and that can also be influenced by the level of antibiotic stress to which the
160 host is exposed. Phage can transduce bacterial genes to other bacteria by generalized or
161 specialized transduction (depending on phages' characteristics, Fig 1F).

162 Input, output and documentation of the model

163 The inputs of each simulation are two text files that define the general parameters and also the
164 ecological setup of the environment (types and numbers of bacteria and/or phage, along with their
165 attributes). The statistics collected at different time points are stored in dictionaries and dataframes
166 (using *pandas*), can be tailored to the experimenter's choice and can be represented visually
167 (using *matplotlib* and *seaborn*) or created as an output file.

168 Random Forest Analysis

169 The Random Forest Analysis is based on simulations performed with the model, covering 3000

random combinations of parameters, with 30 simulated repeats per combination. The output of this cohort of simulations is grouped and resumed in response variables, to which a column with 3000 rows of a random parameter is added (i.e., a choice of a number between 1 and 3). This table is used as input of the randomForest package in R (version 4.6.12), where the *randomForest* function is run with the parameters ntrees set to 10000. The relative importance of each parameter (the percentage increase in minimum squared error, %IncMSE) is assessed using the *importance* function from the same package.

FIG 1. Mechanisms and workflow of the eVIVALDI model. A) Bacterial cells and bacteriophage particles are modelled in a 2-dimensional space, where each (x,y) location holds at most a single bacterial cell and at most a predefined maximum number of phages. The environment ranges from completely well-mixed (liquid), where the contents of each location are randomized at each iteration, to spatially structured, where they are fixed. An intermediate structure is achieved by allowing replication of bacterial cells into a neighbourhood of a given distance. Bacteria and phage can be of different species, and the latter exist as entities either in the environment, where they can infect new hosts, or within hosts, where they either replicate or integrate into their genomes. B) Phage host range is defined in a matrix where each phage has a probability of infecting a given bacterial species. C) Superinfection exclusion is the probability that infection by a given phage aborts when a given type of prophage is present. D) The basal probability of bacterial death can increase by antibiotic exposure or phage infection. Phages decay in function of the period of time spent outside a bacterial host. E) Bacteria compete to reproduce to empty locations, with the fittest bacteria being more likely to produce an offspring. The offspring inherits the traits of the parent cell, but can undergo mutations and is placed into the free location. F) The type of phage infection is determined by the lifestyle of the phage, with virulent following an obligatory lytic cycle, whilst temperate phage can undertake the lytic or the lysogenic cycle following a stochastic decision affected by the density of phages in the environment. The probability of specialized transduction is computed during excision, leading eventually to the incorporation into the phage DNA of a neighboring gene. Generalized transduction occurs before the burst, and a virion has the probability to incorporate random genes from its host, instead of its own DNA. Transduced genes can be used by the subsequently infected bacterial hosts. G) The main cycle of a typical simulation within the model. See complete ODD in Supplementary Material.

195 # Results and Discussion

196 ## Ecology of phage-bacteria interactions in the light of antibiotic and phage therapy

197 Antimicrobial therapies rely on the effectiveness of selective agents to kill sensitive bacteria.
198 Phage therapy involves infection and reproduction of the killing agents, thus extending the ability
199 of standard chemical therapies. We started by investigating if eVIVALDI could reproduce simplified
200 but typical ecological scenarios where sensitive individuals are killed by antibiotics and/or predated
201 by virulent phages, thus promoting the increase in frequency of resistant bacteria. A simple
202 community of two bacterial species, one sensitive and another resistant (either to antibiotic or
203 virulent phage), was simulated in a well-mixed environment, and no new resistant bacterial
204 mutants are allowed to emerge in these simulations. Resistance can be defined as costly, in line
205 with experimental data [41], rendering resistant bacteria less competitive in the absence of
206 selection pressure (Fig S1). However, when either antibiotics (Fig 2A) or phage (Fig 2B) are
207 introduced in the environment, the resistant population rapidly increases to fixation. Predation by
208 phage leads to an initial increase in their numbers, because of the abundance of sensitive bacteria,
209 but also to their subsequent rapid extinction when sensitive hosts become unavailable (Fig 2B). A
210 combined treatment of antibiotics and virulent phages leads to the extinction of both populations
211 because none has the ability to survive both selective pressures (Fig 2C). However, the decrease
212 of the antibiotic sensitive population is slower in the presence of phages because of lower
213 competition from antibiotic resistant cells, which are killed by the phage (Fig S2A).

214 Our model allows to test explicitly the effect of spatial structure on community composition.
215 Antibiotics applied homogeneously in spatially structured communities delay the extinction of the
216 sensitive bacteria in comparison to non-structured environments (Fig S2B). However, antibiotics
217 are more likely to be applied non-homogeneously when environments are structured. The delayed
218 extinction is more pronounced in these conditions leading to long term coexistence between

219 sensitive and resistance bacteria (Fig S3). The effect of phage predation on community dynamics
220 is markedly different between well-mixed and spatially structured environments because the latter
221 decreases dispersion leading to “predation waves” that produce spatial arrangements of dead
222 cells akin to those observed in phage plaque assays (see Fig S4 and Video S1). Ultimately, spatial
223 structure results in delayed extinction of phage sensitive cells (Fig 2E vs Fig 2B). Similar to well-
224 mixed environments, presence of antibiotics and phage in spatially structured environments leads
225 to a much slower extinction of antibiotic resistant bacteria compared to environments with
226 antibiotics but lacking phages (Fig 2F vs Fig 2D). However, the presence of phages and antibiotics
227 in spatially structured environments leads to a faster extinction of antibiotic sensitive populations,
228 compared to well mixed environments (Fig 2F vs Fig 2C, Fig S2C), due to a much less efficient
229 phage predation of their competitors when the environment is structured.

230

231 **Fig 2. Community dynamics driven by antibiotic selection and phage predation.** A small community composed of two different
232 species is subjected to different selective pressures. Bacteria can be sensitive to antibiotics but resistant to phage (in green), or
233 resistant to antibiotics but sensitive to phage (in red). We follow the temporal dynamics and show the populations in their respective
234 colors (the number of free phage in the environment is shown in black). Solid lines indicate mean values for 30 simulations ran with
235 the same parameters and shaded areas show their 95% confidence interval. At the right of each plot is a representative time lapse at
236 3 time points of the lattices for each scenario, where the colors represent each bacterial species and white spaces represent the
237 absence of bacterial cells. In A) and D) antibiotics are applied at the indicated time. In B) and E), virulent phages (10 individual particles)
238 are co-inoculated with the bacteria at time 0. In C) and F), both selective regimes are applied, with antibiotics applied at the indicated
239 time and virulent phage co-inoculated with bacteria at time 0. In A), B) and C), the environment is homogeneous (well-mixed), as in
240 liquid culture. In D), E) and F), the environment is spatially structured. In D) and F) antibiotics are applied homogenously in the
241 structured environment, and in E) and F) each of the 10 phage particles is initially placed randomly in the biofilm. The complete set of
242 parameters for these simulations is show in supplementary data.

243

244 The introduction of mutations in the model, eventually reversing the sensibility to antibiotics or
245 phages, tends to stabilize the bacterial populations (Fig S5A-B). Nevertheless, some populations

still go extinct because of the loss of rare mutants by genetic drift or because no adaptive mutations occurred in the time span. For similar mutation rates, cells resistant to antibiotics increase in frequency faster than the ones resistant to phages, due to differences in the dynamics of the two selection pressures (Fig S5C). Under pressure of antibiotics and phages, double resistant cells emerge only when the mutation rate is very high. The impact of the environmental structure in the dynamics of predation (Fig 2) led us to analyze how it affects the emergence of resistant lineages (Fig 3). Whilst single resistant mutants increase in frequency slower in structured environments (Fig S5D), double mutants resistant to antibiotics and phage are much more likely to emerge (Fig 3A-D) for intermediate rates of mutation (Fig 3E). This is because in structured environments, the rare mutants resistant to antibiotics benefit from the resources available from neighboring dead cells and rise in frequency without contact with phages (that diffuse less efficiently). This increases the span of time available for the acquisition of secondary mutations conferring resistance to phages, especially if the initial number of phages is not very high (Fig S6). Hence, the acquisition of multiple adaptive mutations is more likely to occur in structured environments.

Fig 3. Spatial structure promotes the emergence of multi-resistant bacteria. A-D) Simulations of a single bacterial species, initially sensitive to antibiotics and phage. Lines show 30 replicate simulations with emerging resistant lineages (to one or both selective pressures). Single mutants resistant to phage are shown in red, whilst single mutants resistant to bacteria are shown in green. Double mutant lineages resistant to antibiotics and phage are shown in grey. In A) and B) mutants emerge at a rate of 10^{-4}. C and D) mutants emerge at a rate of 10^{-3}. A and C show dynamics from well-mixed environments. B and D show dynamics from spatially structured environments. E) Percentage of simulations (out of 30) where lineages resistant both to antibiotics and phage have emerged, in either well mixed or spatially structured environments, for all the mutation rates tested (x-axis).

The ability of bacteria to evolve resistance to phage might be futile if phage can also adapt to

overcome the changes in their bacterial hosts [42]. When we allowed bacteria *and* phage to evolve in our simulations (Fig S7A), we observed co-evolutionary arms races similar to both theoretical expectations [33] and experimental observations [43]. Spatially structured environments showed slower co-evolution dynamics and higher variability between simulations than well-mixed ones (Fig S7B). Heterogeneous antibiotics added in structured environments further delayed the co-evolution dynamics (Fig S7C-D), due to the death of a significant part of the bacterial population. Importantly, and as before (Fig 3), surviving bacteria (either resistant to antibiotics or not exposed to lethal concentrations) were able to generate mutants resistant to phages for a longer period of time. This is not only due to the limited diffusion of phage, but also because phages need bacterial hosts to replicate and to generate their own genetic diversity. Thus, a reduction in the number of bacterial hosts due to antibiotic exposure hinders both phage propagation and evolution. This suggests that, in natural environments, multiple stressors might render co-evolutionary arms races less predictable than proposed by theoretical models and experimental settings that assume homogeneous populations and environments.

Lysogeny as a weapon

Contrary to virulent phages, temperate phages may integrate the bacterial host genome and reproduce vertically with it. The lysis-lysogeny decision in our simulations mimics experimental observations [44], and is influenced by the amount of competition faced by the phage: lysogeny is more likely under high viral concentrations or high multiplicity of infection (Fig S8). Since lysogens are protected from further infections by similar phages, due to superinfection exclusion, the environmental concentration of phages in the simulations decreases rapidly with the increase of lysogens (and depending on free phage half-life). When lysogeny occurs mostly at high viral concentration the bacterial population can become extinct before lysogens can arise. Theoretically, this can also result in the extinction of the phage population.

295 When a lysogen invader arrives at a community with resident bacteria sensitive to its prophage,
296 lysis of a small fraction of the invaders can dramatically reduce the population of resident sensitive
297 bacteria. This liberates resources for the lysogenic invaders [13]. eVIVALDI recapitulates previous
298 experimental data on this prophage-as-a-weapon hypothesis [17] (Fig 4a): prophage induction
299 rapidly decreases the sensitive population of residents in the early stages of the process, but
300 lysogenization of the latter rapidly neutralizes this process (because the resident lysogens are
301 now resistant to the phage). Hence, the use of prophages as a biological weapon can provide a
302 decisive advantage for colonizing a new niche, but is rapidly neutralized by lysogenization of
303 competitor bacteria. This is also in agreement with previous theoretical works exploring dynamics
304 of invasion in well-mixed environments, using prophages as a competitive weapon [45].

305 The advantage of lysogens in the colonization of an environment of resident sensitive bacteria
306 was recently demonstrated in the mouse gut and was suggested to depend on the initial ratio
307 between invaders and resident cells [29]. Indeed, our simulations considering different initial ratios
308 of invading lysogens versus resident non-lysogens showed that the latter were more likely to
309 survive as lysogens when more abundant in the beginning of the process (Fig S9). The
310 abovementioned study presented a population-based mathematical model that fitted well most
311 experimental data, but predicted faster initial infection rates than the observed ones. While
312 different parameters can slow down these dynamics (e.g., the burst size of the phage [29]), the
313 spatially structured mouse gastrointestinal tract is likely to interfere with the temporal dynamics of
314 lysogeny. Interestingly, the inclusion of spatial structure in our model, absent from the
315 abovementioned models, led to a slower increase of free viral particles and slower generation of
316 lysogens in the resident strain (Fig 4B-C). This implicates that invading lysogens may be more
317 successful *in vivo* than would have been predicted by *in vitro* studies in well-mixed environments.

318

319 **Fig 4. The role of lysogeny in community dynamics.** A) Genomes from species A (invaders, grey bars) carry an inducible prophage,
320 whereas those of species B (residents, white bars) are initially non-lysogens. Species are co-inoculated at a 1:10 mixture. Phages
321 (black lines) are spontaneously induced from the lysogenic population. These phages infect the sensitive resident population, which
322 may form lysogens that are protected from phages (B*, black bars). Eventually, the resident that are not lysogens become extinct. All
323 bars represent the average of 30 replicate simulations with similar parameters, with the error bars indicating their 95% confidence
324 interval. Data was displayed as in Figure 1 of [17] for comparison. B) Invading lysogens (L, red lines) and resident sensitive cells (S,
325 blue lines) are co-inoculated at a ratio of 1:10. Phages (purple lines) are spontaneously induced and generate new lysogens in the
326 sensitive resident cells (S^L, green lines). Full lines: well-mixed environments. Dashed lines: spatially structured environment. Data was
327 displayed as in Figure 3 of [29] for comparison. C) Emergence of resident lysogens in well-mixed (blue) and in spatially structured
328 (orange) environments during the initial 10 iterations of the simulations shown in B. Shown is the polynomial fit of order 2 for the initial
329 10 iterations, for each of the two types of environment; ANCOVA between the two environments, F=485.5, p=0.

330

331 ## Autotransduction of an antibiotic resistance gene

332 When the phages lysing the resident sensitive cells are able to do generalized transduction, they
333 can transfer adaptive traits back to the invader lysogens (autotransduction [27], Fig 5A). To study
334 this process, we started by demonstrating the adaptive effect of lysogenic conversion in bacteria
335 and how it can impact the competition between different phages (Fig S10). We recreated the
336 conditions for autotransduction within our model, by introducing two strains with similar initial
337 population sizes: a non-lysogenic strain resistant to antibiotics ("residents") and a strain of
338 lysogenic antibiotic sensitive "invaders" (Fig 5A). After initial growth, antibiotics are applied in the
339 environment and, as in the experimental study [27], the invaders survive because they acquire the
340 resistance gene by generalized transduction (Fig 5A-B). The analysis of the bacterial genomes in
341 the simulations indicates multiple successive transduction events from the resistant to the invader
342 cells (Fig 5C). These events are random (i.e., transduction can transfer any part of bacterial DNA),
343 but natural selection results in over-representation of those transferring antibiotic resistance
344 genes. Overall, invaders lead residents to extinction in most simulations (62%), but sometimes
345 residents become lysogens and outcompete invaders (3%). Interestingly, many simulations

346 exhibited coexistence of lysogenic invaders and lysogenic residents (22%, Fig 5D), and a few
347 showed extinction of all bacterial populations (13%).

348

349 **Fig 5. Simulation of autotransduction.** A) Representation of the autotransduction events. We created a multispecies community akin
350 to the experimental work of [27], where the invader lysogenic species (red) is sensitive to antibiotics and the resident non-lysogenic
351 species (green) is resistant to antibiotics but sensitive to the phage of the invaders. B) Temporal dynamics of a typical simulation
352 leading to the survival of the invaders. The black line indicates the number of phages in the environment and the time of application of
353 antibiotics is indicated with the grey line. C) Samples of genomes in the population at two different time points of the simulations of
354 panel B. Before antibiotics (*t=2*), the genomes of the resident population (green) carry the resistance trait (orange marker). The invader
355 population (red) is not resistant (grey marker indicates sensitivity to drugs). After the application of antibiotic (*t=10*) most of the invaders
356 have the original prophage and a random sequence of bacterial DNA transduced from the resident cells (other ellipses). D) Outcome
357 of 100 simulations.

358

359 eVIVALDI includes many complex stochastic mechanisms and it is not straightforward to
360 empirically disentangle the importance of each in the final outcome. Therefore, we used a machine
361 learning approach, Random Forest Analysis (RFA, see Methods), to quantify the importance of
362 the mechanisms driving the increase of the population of invaders (Fig 6A, Table S1). We focused
363 on the percentage increase in minimum squared error (MSE) associated with each variable in the
364 simulation. Generalized transduction had the strongest effect in the efficiency of autotransduction
365 (86% increase in mean square error [MSE], Fig 6B), whilst specialized transduction was almost
366 negligible (3% increase in MSE). Autotransduction also improved with higher probability of
367 adsorption (44% increase in MSE) and infection distance (i.e., the maximum distance between a
368 bacterium and a phage still allowing infection, 70% increase in MSE), because they increase the
369 reach and efficiency of infection by phage and, subsequently, the likelihood of generalized
370 transduction (Fig 6C, Fig S11B). In contrast, when the decision to enter lysogeny (49% increase
371 in MSE) can be made with high probability for relatively low viral concentrations, the resident

population proliferates (Fig S11D and Fig S11I for the lysis-lysogeny decision functions explored with the RFA). The importance of the remaining parameters is detailed in Fig S11.

Fig 6. Identification of the main mechanisms affecting the rate of autotransduction of an antibiotic resistance gene using Random Forest Analysis. A) Analysis is based on 3000 randomized combinations of parameters and 30 repeated simulations for each combination). Parameters with a higher % in increased minimum square error have a higher importance for the measured outcome: the median of the final relative frequency of the invader. A random parameter (in grey) was included in the analysis to provide a baseline reference of importance. B-C) The directionality of the impact of two parameters is assessed by plotting the frequency of the invader population at the end of the simulation (across all simulations), in function of the parameter of interest (the other parameters are shown in Fig S11). In the left y-axis, and as strip plot of grey dots, is the distribution of the frequency of the invader population in all simulations. In the right y-axis, and as red dots and lines, is the median of this frequency across the simulations.

To better understand the relationship between two of the most important parameters, generalized transduction and probability of phage attachment, we explored their space of parameters at a higher resolution than before (Fig 7A), while fixing all other parameters. We found that a critical combination of high adsorption efficiency and high (but not too high) probability of generalized transduction is required for the survival of invaders (red region). The survival of the resident population is the most likely outcome when rates of transduction and/or infection are low, but also when all phages engage in generalized transduction (100% probability of generalized transduction), because no viable particles are released in the environment (green region). The space of parameters leading to coexistence (yellow region) separates the region leading to the overrepresentation of the invaders from the one leading to the overrepresentation of the resident species (see also Fig S12).

The study revealing autotransduction focused on the process of gene acquisition by the invaders and did not address the possibility of co-existence [27]. We thus experimentally addressed the

397 prediction of co-existence by co-culturing in liquid media supplemented with chloroamphenicol two
398 strains of *Staphylococcus aureus*: a lysogenic strain (JH944, “invaders”) sensitive to the antibiotic
399 and a non-lysogenic strain (JH938, “residents”) resistant to the antibiotic (see Methods). The
400 majority of the clones isolated at the end of the experiment are from the JH944 background, and
401 these are now resistant to chloroamphenicol, indicating the acquisition of the resistance gene from
402 the resident bacteria by autotransduction. However, in all 3 replicates a subpopulation of JH938
403 was observed to coexist with the invader strain (Fig 7B), confirming our predictions.

404 Importantly, coexistence is strictly dependent on the generation of lysogenic variants of the
405 resident bacteria, being suppressed when we performed simulations without the generation of
406 new lysogens (Fig S13A-D). This is experimentally corroborated with the observed release of
407 phage particles from the surviving resident clones at the end of the co-culture, when these are
408 exposed to Mitomycin C (Fig 7B, see Methods). This confirms that lysogenization of the resident
409 bacteria is the mechanism responsible for the coexistence between the two strains.

410 Our simulations further suggest that structured environments (Fig S12A) provide an additional
411 region, for extremely high rates of transduction, where coexistence is prevalent (upper regions in
412 Fig S12B-C). These high rates are biologically implausible for viable phages, but not for defective
413 phages or for gene transfer agents [46]. Finally, extinctions of both strains were more frequent
414 when the probability if adsorption was high and transduction was low, suggesting that an inducible
415 phage that is highly infective but a poor transducer is more likely to lead to the collapse of both
416 the invaders and the antibiotic resistant populations. The likelihood of double extinctions is higher
417 in structured environments (Fig S12A, Fig S14 and Fig S15) or in the absence of lysogenization
418 of the resident bacteria (Fig S13E-F and Fig S16). Our results suggest that ecological interactions
419 between strains invading communities of susceptible bacteria can be very diverse, depending on
420 the rates of infection, transduction, lysogenization and population structure.

421

422 **Fig 7. The combined role of probability of adsorption and generalized transduction for the autotransduction of an antibiotic**
423 **resistance gene.** The simulation scenario is similar to Fig 6. **A)** The heatmap represents the likelihood of the outcome of the
424 simulations in function of the two parameters. The color scale ranges from green (100% of the final population composed resident
425 bacteria) to red (100% of the final population composed of invader bacteria), with yellow regions indicating cases where coexistence
426 is the outcome more likely to occur in the timeframe of the simulations. 30 repeat simulations were performed for each combination of
427 parameters, and their median value is used to construct the heatmap. When both populations went extinct, this was either ignored to
428 compute the median (if it occurred in less than 50% of the cases), or was marked as grey (otherwise). **B)** Co-cultures of a lysogenic
429 choloroamphenicol sensitive strain of *S. aureus* (JH944, "invaders") and a non-lysogenic choloroamphenicol resistant strain (JH938,
430 "residents") indicate coexistence between the two strains at the end of the experiment. Y-axis shows the percentage of colonies with
431 a given genotype (out of 127 in total). Boxplots and dots represent the data for 3 independent replicates of the experiment.

432

433

Conclusion

Individual-based modelling is providing novel ways to analyze and predict the behavior of microbial systems [39]. Our novel approach integrates multiple and different bacterial species, phages, environmental structures and ecological conditions to explore different aspects of bacteria-phage interactions: temporal changes in community composition (e.g., between lysogens and non-lysogenic bacteria), the concurrent effects of mechanisms of infection, lysogeny, and transduction, and their consequences for the genomic composition of each individual bacteria and phage. To the best of our knowledge, no other theoretical or computation model integrates these different scales of bacteria-phage interactions. This has allowed us to characterize and quantify key ecological components, such as structured environments, in the dynamics emerging from these interactions.

Nonetheless, models are grounded on simplifying assumptions to make biological systems more tractable. This facilitates pinpointing the relevance of certain mechanisms or agents, but may result in misleading over-simplifications of the system. One major difference between biological systems and our model concerns the number of cells which, due to computational reasons, is lower than the one typically used in experimental settings. Even though our results are qualitatively similar to experimental and/or other theoretical works, this difference may affect the quantitative results. The decreased effective population size (and the consequent increase in the effect of drift) requires that certain rates (e.g., mutation or transduction rates) are simulated at higher values, in order to increase the probability of detecting such events. Another limitation lies in the characterization of the environment. Even if we allow for different levels of structure, environments are spatially and temporally constant throughout the simulations, which might not always be the case in nature. This can change the dynamics of propagation of phages, and lead to subpopulations specialized for different spatial niches. A third limitation of the model lies on the

458 lack of a true physiological description of the bacteria. We assume that phages can infect bacteria
459 at any time, but phage infectivity is known, in some cases, to depend on whether its bacterial host
460 is in exponential or stationary phase [47]. In other cases, a stochastic or induced persistence state
461 in bacteria allows the population to maintain alive a sensitive subpopulation [48]. This can lead to
462 a slowdown or complete halt of infection, particularly in structured environments. Nevertheless, it
463 is important to underline that the model was designed to be easily extensible and further assimilate
464 new mechanisms. Some that are already implemented but not thoroughly explored here include
465 phage resistance based on adaptive immunity (e.g., CRISPR-Cas [32,49]) or mutations affecting
466 the phage host range [50].

467 One of the major conclusions of this work is that spatial structure affects the dynamics of bacterial
468 populations in the face of antibiotic exposure, phage predation or a combination of both. Whilst
469 combining phages and antibiotics is one of the proposed strategies for the clinical use of phage
470 [51,52], we show here that the emergence of bacteria resistant to both stressors can be enhanced
471 by structured environments, particularly when antibiotics are not homogeneously distributed, as
472 seems common in natural settings [53,54].

473 Adaptation of bacterial cells can also be driven by temperate phage, and we explore in detail how
474 autotransduction can promote the spread of antibiotic resistance, quantifying the relative
475 importance of different mechanisms for this outcome. Importantly, we predict that different
476 community outcomes (coexistence and extinction) can occur by modulating the efficiency of
477 phages' infection, lysogeny and transduction, as well as the structure of the environment. We
478 experimentally confirm the emergence of co-existence between strains of *S. aureus* in well mixed
479 environments, but it would be interesting in the future to modulate the efficiency of phage infection
480 and of generating transducing particles. Furthermore, experimentally assessing the predicted role
481 of structured environments in generating different conditions for co-existence and extinction will
482 be important. It would also be crucial to explore how co-existence between virulent phage and

483 prophage influences the outcomes of a combined treatment with phage and antibiotics. Exploring
484 these and other ecological settings is key to understand which factors impact the efficiency of
485 phage therapy, and their evolutionary consequences for bacterial populations.

486

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492

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645

Fig 1

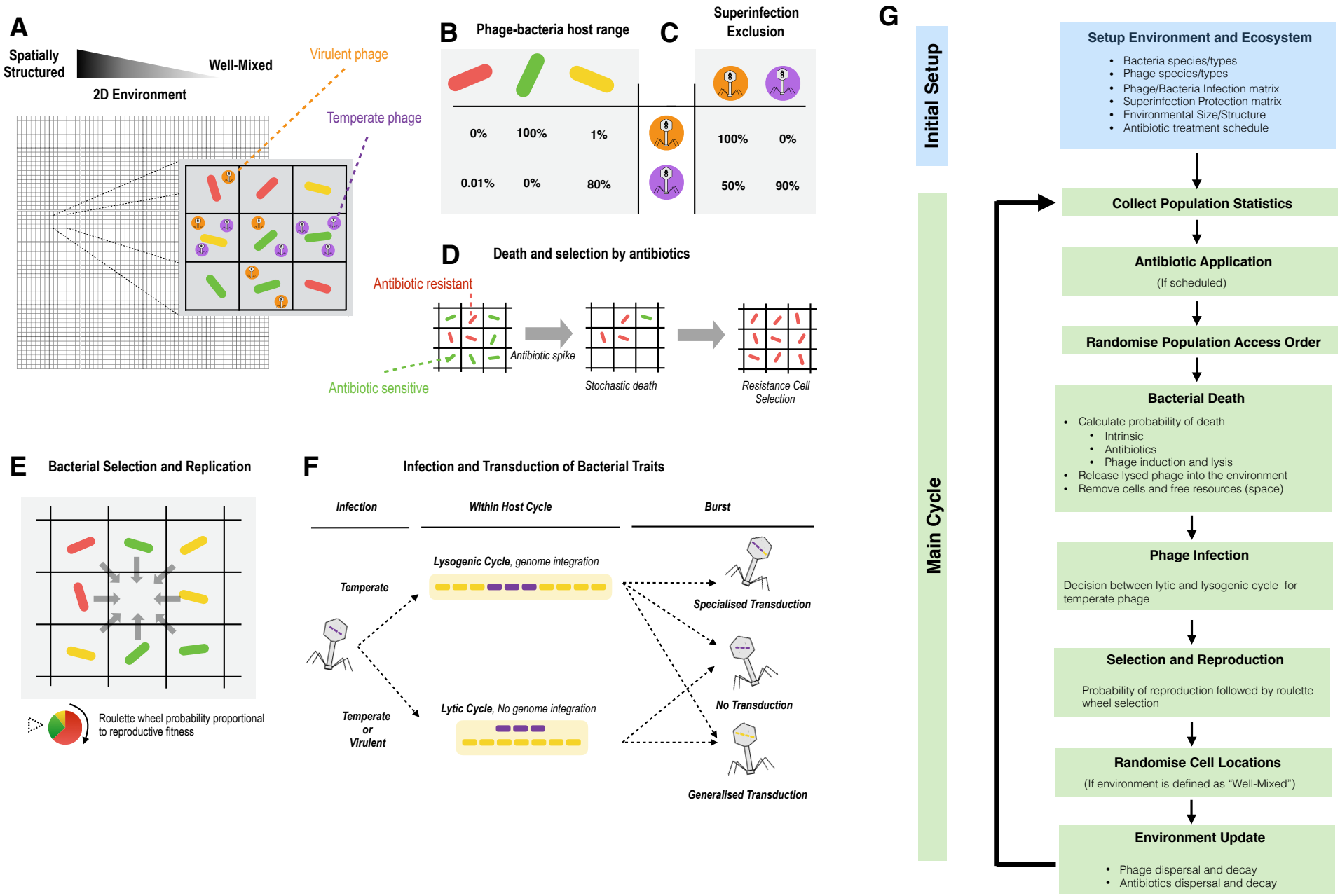

A
Spatially Structured
Well-Mixed
2D Environment
Virulent phage
Temperate phage
B
Phage-bacteria host range
0%
100%
1%
0.01%
0%
80%
C
Superinfection Exclusion
100%
0%
50%
90%
D
Death and selection by antibiotics
Antibiotic resistant
Antibiotic sensitive
Antibiotic spike
Stochastic death
Resistance Cell Selection
E
Bacterial Selection and Replication
Roulette wheel probability proportional to reproductive fitness
F
Infection and Transduction of Bacterial Traits
Infection
Within Host Cycle
Burst
Lysogenic Cycle, genome integration
Temperate
Specialised Transduction
No Transduction
Temperate or Virulent
Lytic Cycle, No genome integration
Generalised Transduction
G
Initial Setup
Setup Environment and Ecosystem
Bacteria species/types
Phage species/types
Phage/Bacteria Infection matrix
Superinfection Protection matrix
Environmental Size/Structure
Antibiotic treatment schedule
Main Cycle
Collect Population Statistics
Antibiotic Application
(If scheduled)
Randomise Population Access Order
Bacterial Death
Calculate probability of death
Intrinsic
Antibiotics
Phage induction and lysis
Release lysed phage into the environment
Remove cells and free resources (space)
Phage Infection
Decision between lytic and lysogenic cycle for temperate phage
Selection and Reproduction
Probability of reproduction followed by roulette wheel selection
Randomise Cell Locations
(If environment is defined as "Well-Mixed")
Environment Update
Phage dispersal and decay
Antibiotics dispersal and decay

Fig 2

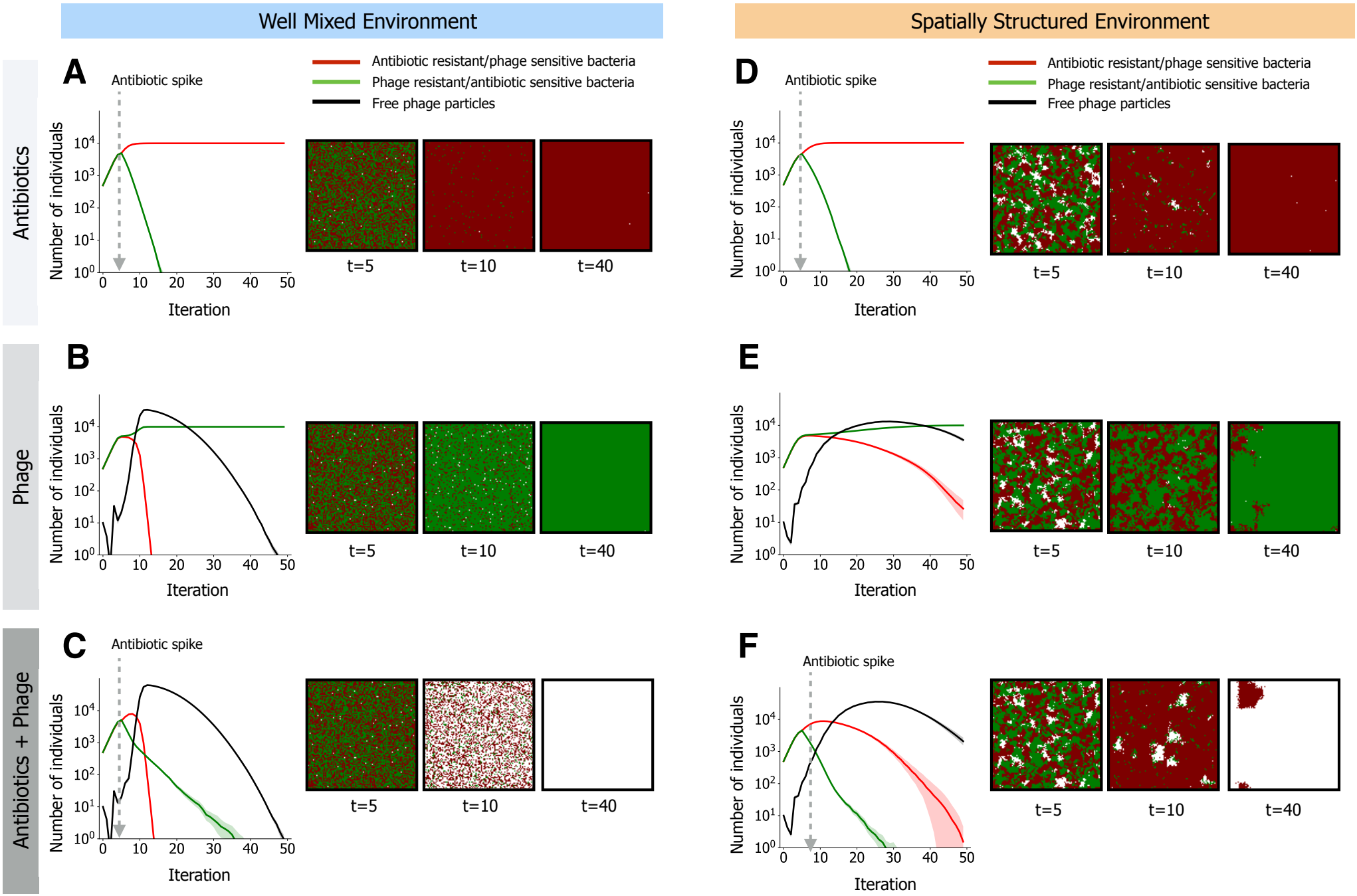

Well Mixed Environment
Spatially Structured Environment
Antibiotics
Phage
Antibiotics + Phage
A
B
C
D
E
F
Antibiotic spike
Antibiotic resistant/phage sensitive bacteria
Phage resistant/antibiotic sensitive bacteria
Free phage particles
Number of individuals
Iteration
t=5
t=10
t=40

Fig 3

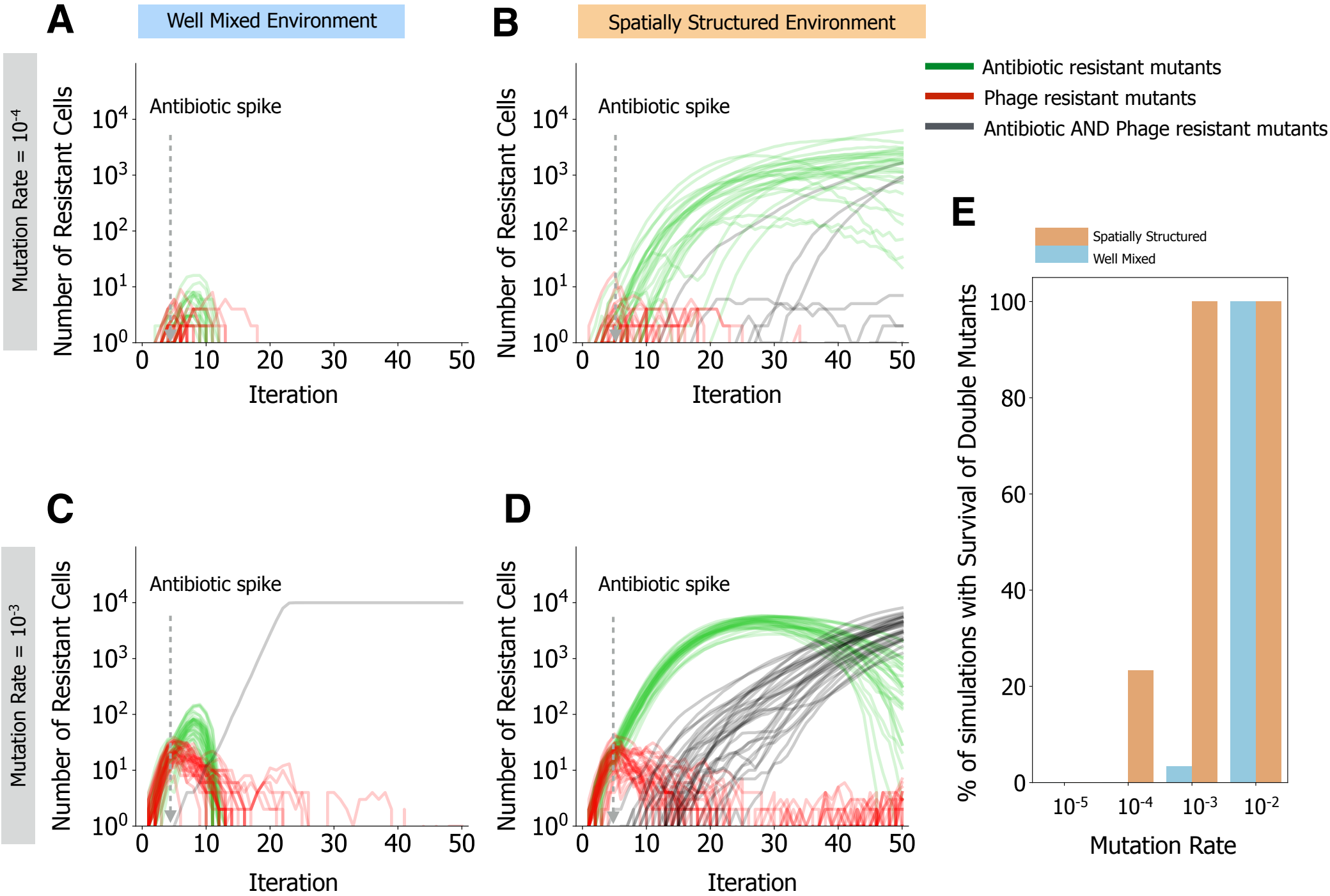

A
Well Mixed Environment
B
Spatially Structured Environment
Antibiotic resistant mutants
Phage resistant mutants
Antibiotic AND Phage resistant mutants
Mutation Rate = 10^{-4}
Mutation Rate = 10^{-3}
Number of Resistant Cells
Antibiotic spike
Iteration
C
D
E
Spatially Structured
Well Mixed
% of simulations with Survival of Double Mutants
Mutation Rate

Fig 4

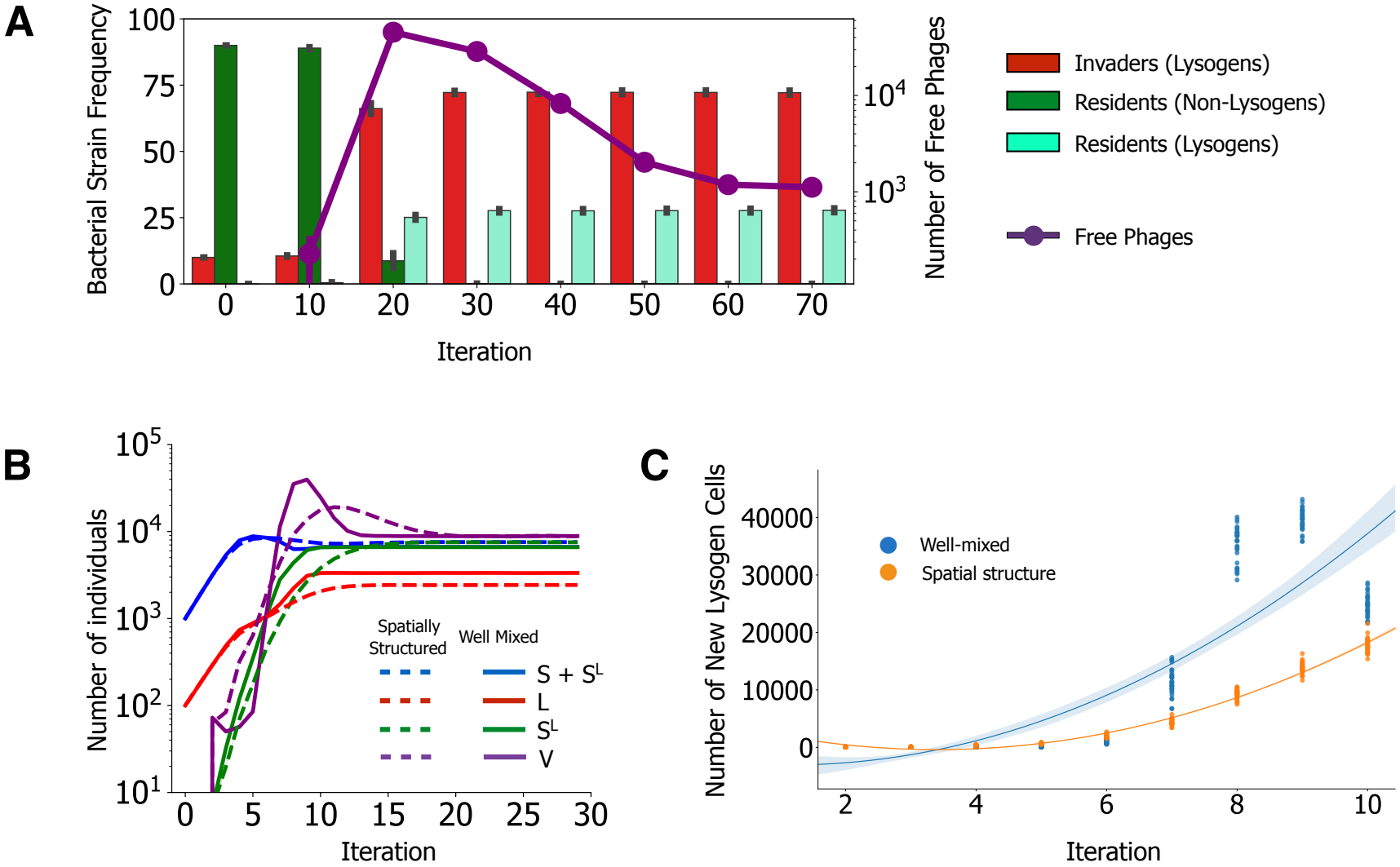

A
Bacterial Strain Frequency
100
75
50
25
0
0
10
20
30
40
50
60
70
Iteration
10^4
10^3
Number of Free Phages
Invaders (Lysogens)
Residents (Non-Lysogens)
Residents (Lysogens)
Free Phages
B
Number of individuals
10^5
10^4
10^3
10^2
10^1
0
5
10
15
20
25
30
Iteration
Spatially Structured
Well Mixed
S + S^L
L
S^L
V
C
Number of New Lysogen Cells
40000
30000
20000
10000
0
2
4
6
8
10
Iteration
Well-mixed
Spatial structure

Fig 5

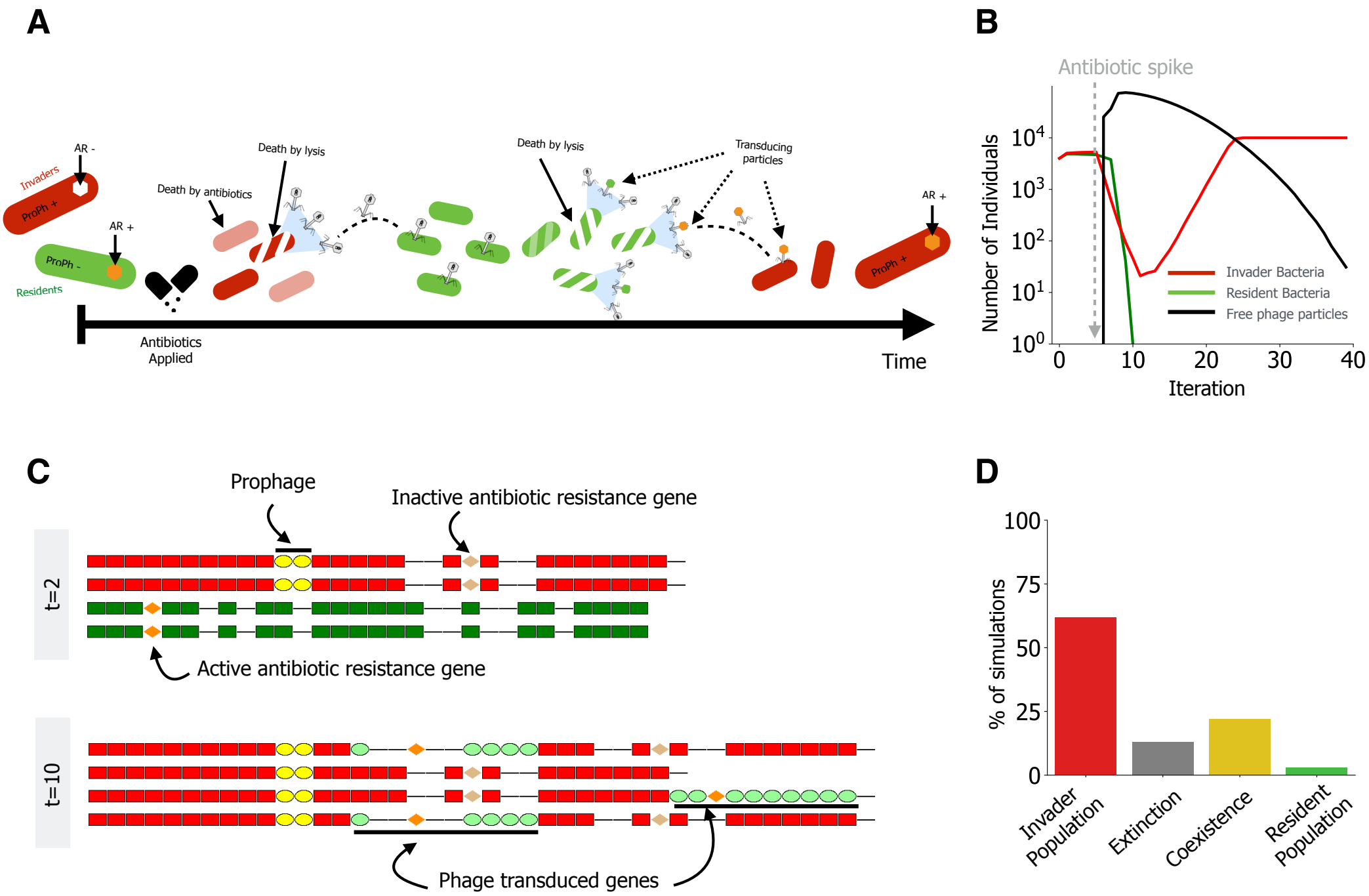

A
Invaders
ProPh +
AR -
ProPh -
AR +
Residents
Death by antibiotics
Death by lysis
Death by lysis
Transducing particles
AR +
ProPh +
Antibiotics Applied
Time
B
Antibiotic spike
Number of Individuals
10^4
10^3
10^2
10^1
10^0
0
10
20
30
40
Iteration
Invader Bacteria
Resident Bacteria
Free phage particles
C
Prophage
Inactive antibiotic resistance gene
t=2
Active antibiotic resistance gene
t=10
Phage transduced genes
D
% of simulations
100
75
50
25
0
Invader Population
Extinction
Coexistence
Resident Population

Fig 6

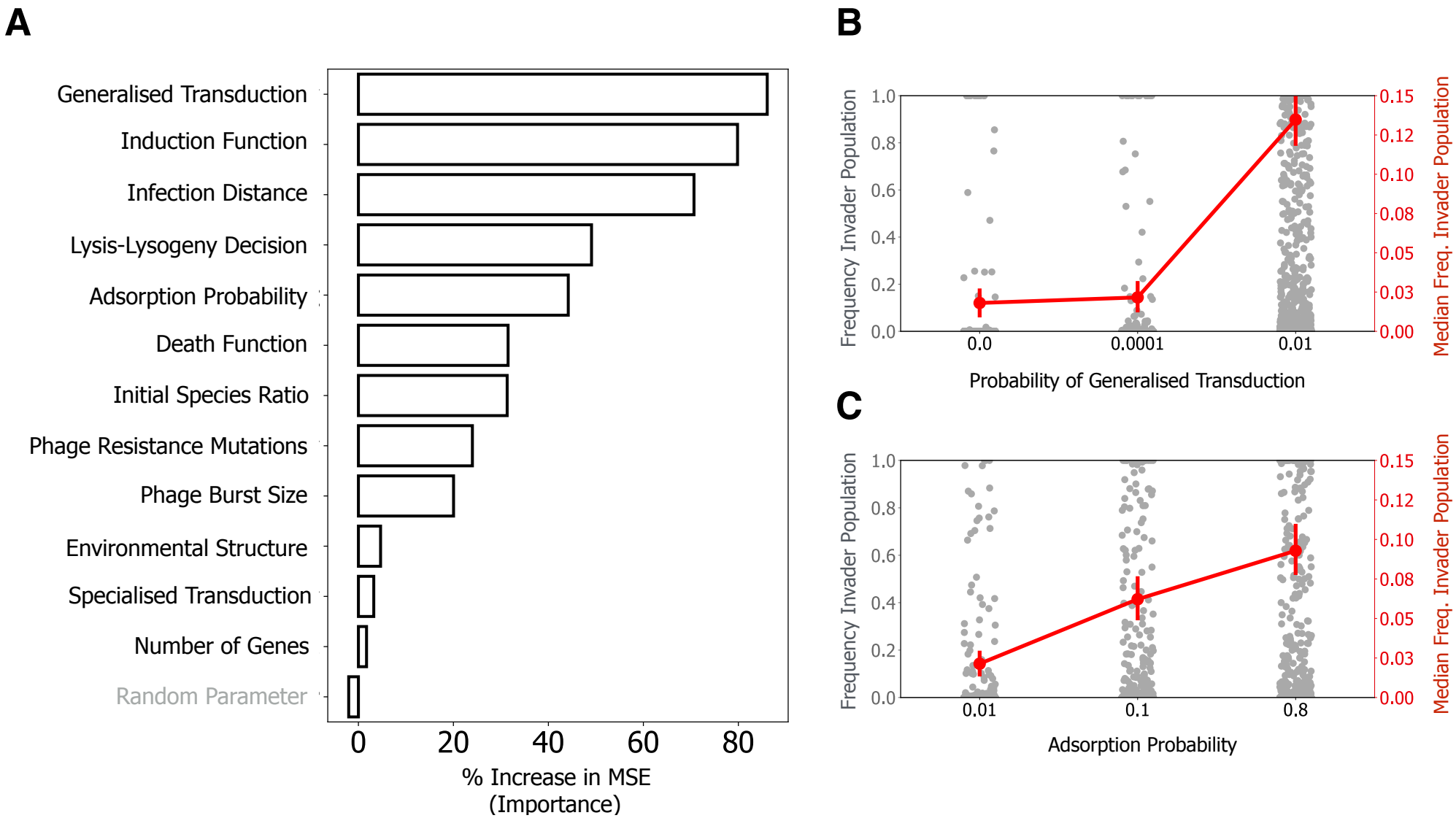
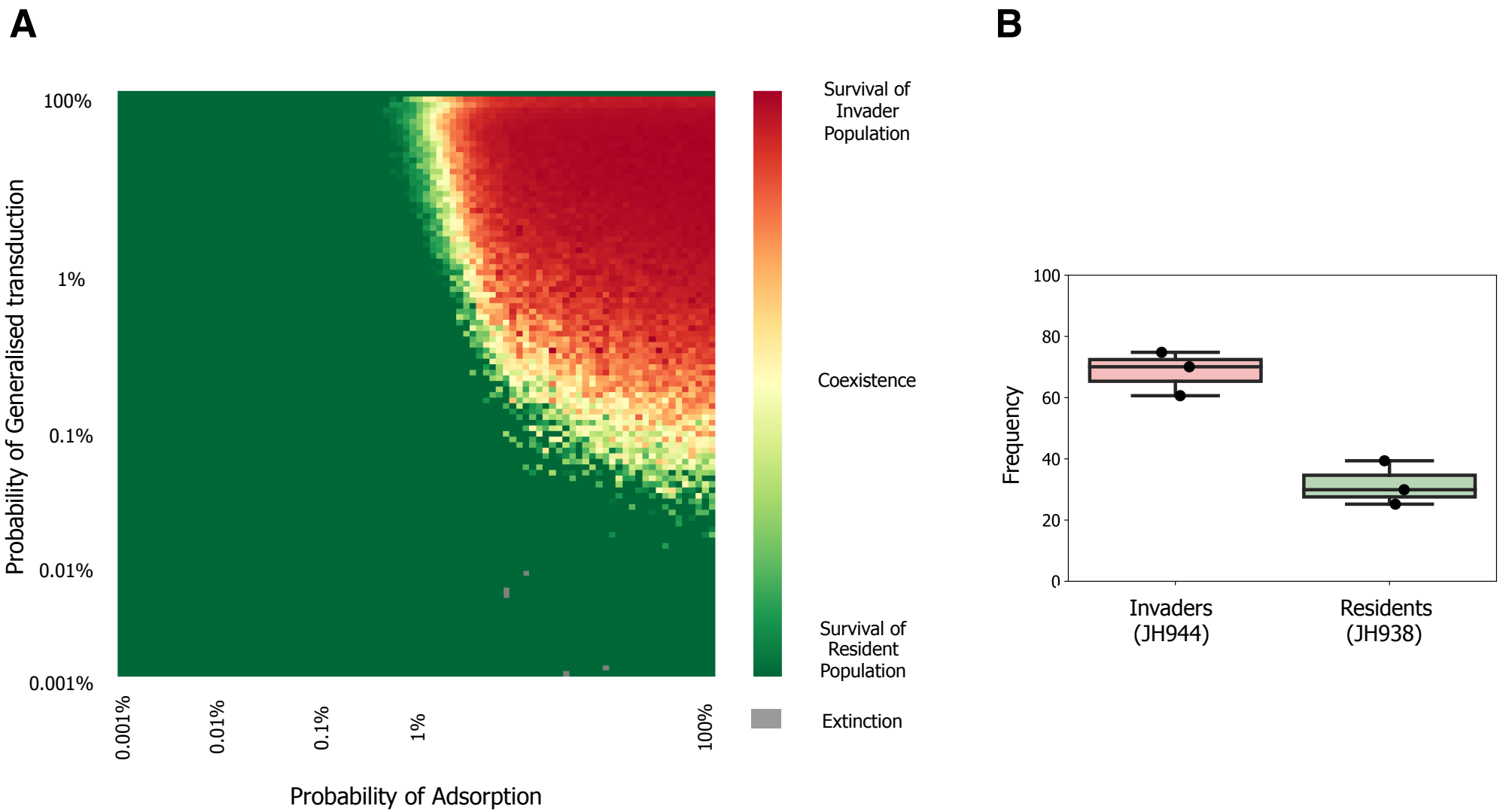

A
Generalised Transduction
Induction Function
Infection Distance
Lysis-Lysogeny Decision
Adsorption Probability
Death Function
Initial Species Ratio
Phage Resistance Mutations
Phage Burst Size
Environmental Structure
Specialised Transduction
Number of Genes
Random Parameter
0
20
40
60
80
% Increase in MSE
(Importance)
B
Frequency Invader Population
Median Freq. Invader Population
1.0
0.8
0.6
0.4
0.2
0.0
0.15
0.12
0.10
0.08
0.05
0.03
0.00
0.0
0.0001
0.01
Probability of Generalised Transduction
C
Frequency Invader Population
Median Freq. Invader Population
1.0
0.8
0.6
0.4
0.2
0.0
0.15
0.12
0.10
0.08
0.05
0.03
0.00
0.01
0.1
0.8
Adsorption Probability

Fig 7


A
100%
1%
0.1%
0.01%
0.001%
Probability of Generalised transduction
0.001%
0.01%
0.1%
1%
100%
Probability of Adsorption
Survival of Invader Population
Coexistence
Survival of Resident Population
Extinction
B
100
80
60
40
20
0
Frequency
Invaders (JH944)
Residents (JH938)