The ecological consequences and evolution of retron-mediated suicide as a way to protect *Escherichia coli* from being killed by phage

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Author Contributions: BAB, RG, and BRL contributed to the experimental design, modelling, and analysis of the experiments as well as the writing of this report. JAM contributed to the execution of the experiments and the analysis of their results.

Competing Interests Statement: The authors have no competition interests to declare.

Abstract

In 1984, retrons were described as DNA sequences that code for a reverse transcriptase and a unique single-stranded DNA/RNA hybrid called multicopy single-stranded DNA (msDNA). It would not be until 2020 that a function was shown, when compelling evidence was presented that retrons can protect bacteria from infections by lytic phage via an abortive infection mechanism. When infected with the virulent mutant of the phage lambda, λ^{VIR}, and to a lesser extent other phages, a retron designated Ec48 is activated, the *Escherichia coli* bearing this element dies and the infecting phage is lost. With the aid of a mathematical model, we explored the *a priori* conditions under which retrons will protect bacterial populations from invasion by phage and when retron-bearing bacteria will evolve in populations without this element. Using *E. coli* with and without Ec48, and λ^{VIR} we estimated the parameters of this model and tested the hypotheses generated from our analysis of its properties. Our models and experiments suggest cells expressing retron-mediated abortive infection have no advantage over cells lacking this system as a defense against phage, and we question whether the protection against phage infection is the unique selective force responsible for their evolution in bacteria.

Introduction

Retrons, DNA sequences that code for a reverse transcriptase and a unique single-stranded DNA/RNA hybrid called multicopy single-stranded DNA (msDNA), were discovered in 1984 (1) and were the first example of a reverse transcriptase coded for by bacteria (2, 3). These elements were initially found in *Myxococcus xanthus,* but subsequently have been found in a number of bacterial species, including *Escherichia coli* (4, 5). Like CRISPR-Cas, retrons are being employed for genome engineering (5-7), and are capable of doing editing tasks that cannot be done by CRISPR-Cas (8). Also like CRISPR-Cas the function of retrons was not determined for decades after their discovery and molecular characterization (9). For retrons, the identification of function came in 2020 when Millman and collaborators presented compelling evidence that retrons can defend *E. coli* from infections with lytic phages (10).

The mechanism by which retrons protect bacteria from lytic phages is known as abortive infection (abi); the infected cell dies with its infecting phage (11). From an evolutionary perspective, abi is intriguing. Phage-defense systems like envelope resistance, restriction-modification, and CRISPR-Cas are to the advantage of the individual bacteria expressing them. This is not the case for abortive infection; the defense against phage is through suicide and thereby not to the advantage of the individual bacteria expressing this defense mechanism. To account for the evolution of abortive infection mechanisms, it has been postulated that abi is an altruistic trait which provides an advantage to the collective, clonal population (12, 13). Individual cells that commit suicide due to phage infection prevent these viruses from infecting and killing other, genetically identical, members of their population.

Using agent-based models and experiments with *E. coli* and its phages, Fukuyo and colleagues and Berngruber and colleagues (12, 13), provide a theoretical basis for and experimental support for this altruistic suicide hypothesis for the ecological role and evolution of abortive infection. They show that in physically structured habitats, abortive-infection can successfully protect populations against invasion by phage, and phage-mediated selection can lead to the evolution of abortive infection.

Here we explore the conditions under which, by abortive infection, retrons can protect bacterial populations from invasion by lytic phage and test the hypothesis that defense against phage is the selective force responsible for the evolution of retrons in bacterial populations. Using a mathematical model of the population and evolutionary dynamics of bacteria and phage in mass culture, we explore the a priori conditions under which retron-mediated abortive infection can protect populations of bacteria from invasion by lytic phage, and that phage-mediated selection will enable retrons to become established and maintained in bacterial populations. Using E. coli K12 bearing the retron Ec48 described by Millman and colleagues, (10) and a virulent mutant of the phage lambda, λ^{VIR}, we estimate the parameters of this model and test the validity of the predictions generated from our numerical analysis of the populations' properties.

The results of our experiments support the proposition that both in liquid and physically structured habitats, retrons can protect populations of bacteria from infection by phage. Our models and experiments also indicate that the conditions for retrons playing this ecological role

are restrictive; the abortive infection mechanism must be nearly perfect, and there cannot be retron-free bacteria that can support the growth of the phage. As anticipated by our models and the theory and experiments reported in (12, 13), in mass, liquid culture unless their carriage provides a marked selective advantage, even in the presence of phage, retrons will not become established in populations of bacteria susceptible to these viruses. Contrary to the abi system studied in (12), in the physically structured habitat of soft agar, by abortive infection alone retrons cannot become established in populations not coding for this element. Moreover, in both liquid and soft agar culture, in the presence of λ^{VIR} envelope resistance mutants emerge in retron-bearing and retron-free *E. coli* populations emerge and become the dominant populations of bacteria, thereby confounding the role of abortive infection as a defense against phage infection.

Results

A model of retron-mediated abortive infection

To build the theoretical background to generate our hypotheses, design our experiments, and to interpret their results, we generated a mathematical model. This model is based on the interactions of the populations illustrated in Figure 1, along with the definitions and assumptions detailed in the methods section and parameters defined in Supplemental Table 1. In accord with this model, the rates of change in the densities of bacteria and phage and the concentration of the limiting resource are given by the system of time-dependent, coupled differential equations listed in Supplemental Equations (1) – (7).

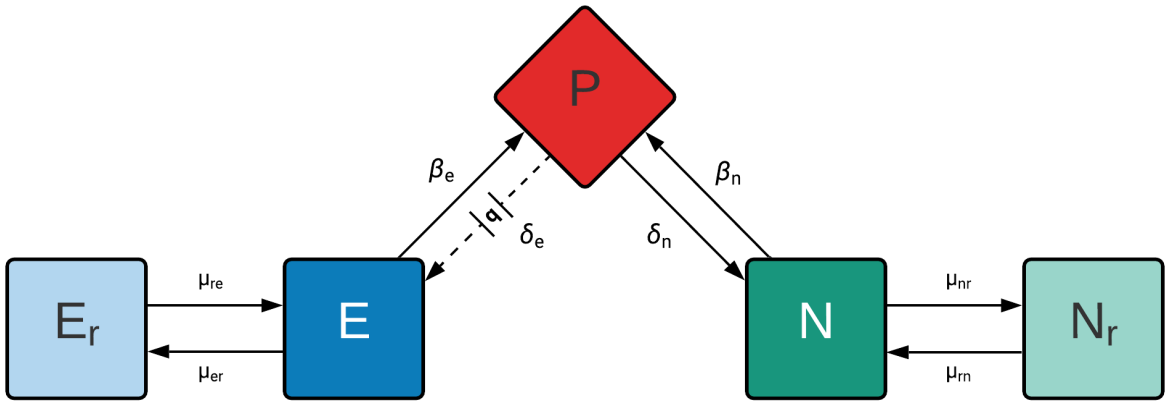


Figure 1. Diagram of a model of the population and evolutionary dynamics of lytic phage and bacteria with and without a retron-mediated abortive infection mechanism. There is a single population of lytic phage, P; a phage-sensitive retron-encoding (retron$^+$) population, E; an envelope resistant retron$^+$ population, E_r; a phage-sensitive (retron$^-$) population, N; and an envelope resistant retron$^-$ population, N_r. The phage adsorbs to the N and E bacteria with rate constants, δ_n and δ_e (ml·cells/hour), respectively. The phage replicates on the N population with each infection produced β_n phage particles, the burst size. A fraction q ($0 \leq q \leq 1$) of the phages that adsorb to E population are lost and thus do not replicate. The remaining (1 - q) of infections of E produce β_e phage particles. At rates μ_{nr} and μ_{er} per cell per hour, the bacteria transition from their respective sensitive to resistant states, and at rates μ_{rn} and μ_{re} they transition from the resistant to their respective phage sensitive states.

96 Computer simulations of the population densities in retron-mediated protection against phage
97 infection

98 We open this consideration of the population and evolutionary dynamics of retron-mediated
99 abortive infection with an analysis of the ability of retrons to provide populations protection from
100 predation by lytic phage. For this theoretical and the subsequent experimental considerations of
101 retron-mediated abi, we present the predicted and observed densities of bacteria and phage
102 when their populations first encounter each other, time 0, and at 24 hours (Figure 2). We
103 performed an analysis to determine the minimum efficacy of retron-mediated abortive infection
104 needed to protect populations from phage infection based on a range of values of abi
105 effectiveness (q of 0.1 to 1.0, with steps of 0.01). We found that at least 98% of the infection
106 events had to be aborted by the retron to protect the population from phage (Figure S1). In other
107 words, q must be at least 0.98 for retron-mediated abi to be protective. With these results in
108 mind, we selected a q value of 1.00 and 0.95, to illustrate abortive infection success and failure,
109 respectively Figures 2A and 2B.

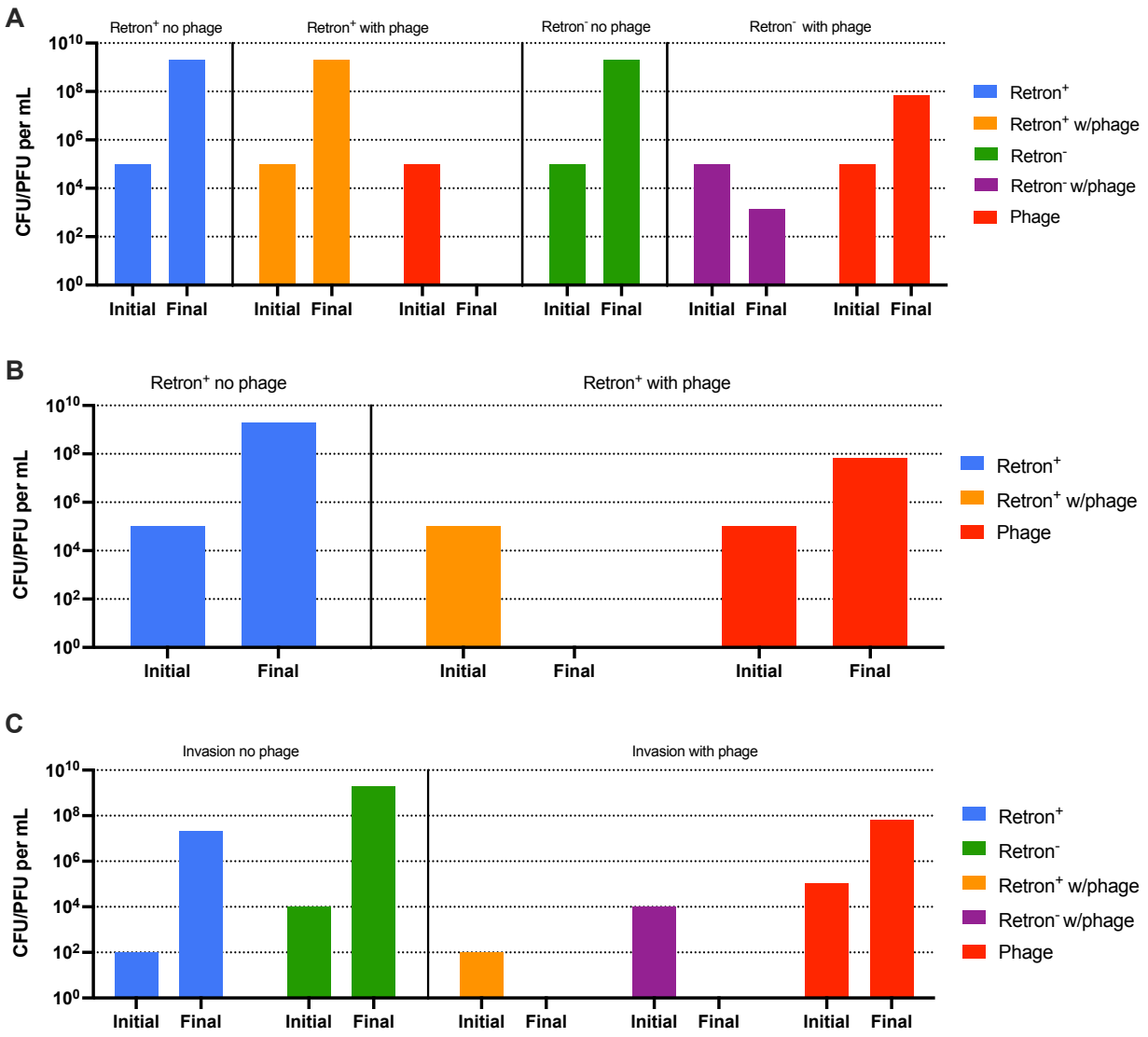


Figure 2. Computer simulation results without envelope resistance. Changes in the densities of a retron$^+$ bacterial population in the absence (blue) and presence (orange) of phage (red) and a retron$^-$ bacterial population in the absence (green) and presence (purple) of phage at 0 (Initial) and 24 hours (Final). Unless otherwise stated, the parameters used to simulate were: k=1, e= 5×10^{-7} µg/cell, $v_e = v_n = 2.0\ h^{-1}$, $\delta_e=\delta_n=2\times10^{-7}\ h^{-1}cell^{-1}$, $\beta_a=\beta_n=60$ phages/cell, $\mu_{nr}=\mu_{rn}=\mu_{er}=\mu_{re}=0$ (not allowing for resistance generation). **A-** A completely effective (q=1.00) retron positive (retron$^+$) bacterial population in the absence and presence of phage. **B-** An incompletely effective (q=0.95) retron positive (retron$^+$) bacterial population in the absence and presence of phage. **C-** Invasion of a completely effective (q=1.00) retron$^+$ population into a high density of retron$^-$ bacteria in the absence and presence of phage.

As can be seen in Figure 2A, a completely effective retron-mediated abi defense system (q=1.00) is able to protect a population of retron$^+$ bacteria from predation by phage. By 24 hours, the phage population is gone and the retron$^+$ population is at its maximum density. When the retron$^-$

populations are confronted with phage, by 24 hours the bacteria are present at a low density and there is a substantial density of free phage. The ability of the retron to prevent the ascent of the phage and protect the bacterial population is critically dependent on the efficacy of retron-mediated abortive infection (Figure 2A, 2B). When a mixed initial population of the retron$^+$ and retron$^-$ bacteria are confronted with phage, the phage replicates on the retron$^-$ cells and the phage population increases in density. In this case, the retron$^+$ bacteria are lost entirely (Figure 2C).

In Figure 3, we present the results of our experimental tests of the retron protection hypotheses presented in Figure 2 using the retron$^+$ *E. coli* Ec48 (10) and a lytic mutant of the phage lambda, λ^{VIR}. As a retron$^-$ control, we use a λ^{VIR} sensitive *E. coli* C. As anticipated from the model (Figure 2A), when confronting the retron$^+$ population by 24 hours, the λ^{VIR} population is gone or nearly so, and the bacterial density is at the level of a phage-free control (Figure 3A). The results of the control experiment with the λ^{VIR} and the retron$^-$ sensitive strain are inconsistent with the prediction of the model (Figure 2B). As anticipated by the model, the phage density increased over 24 hours, but contrary to what is expected from the model (Figure 2A), the bacteria were not lost, but rather increased to the density anticipated in the absence of phage (Figure 3B). Also inconsistent with the prediction of the model (Figure 2C), in the presence of phage the retron$^+$ population does not become extinct when initially rare relative to the retron$^-$ population. One possible reason for this, is that the bacteria recovered at 24 hours from the retron$^-$ population are resistant to λ^{VIR}. To test this hypothesis, we employed the cross-streak method on colonies isolated at 24 hours to determine their susceptibility to λ^{VIR} (Table S3). By this criterion, the vast majority of the initially sensitive retron$^-$ bacteria recovered at 24 hours are resistant to λ^{VIR}. This is also the case for the retron$^+$ bacteria recovered at 24 hours.

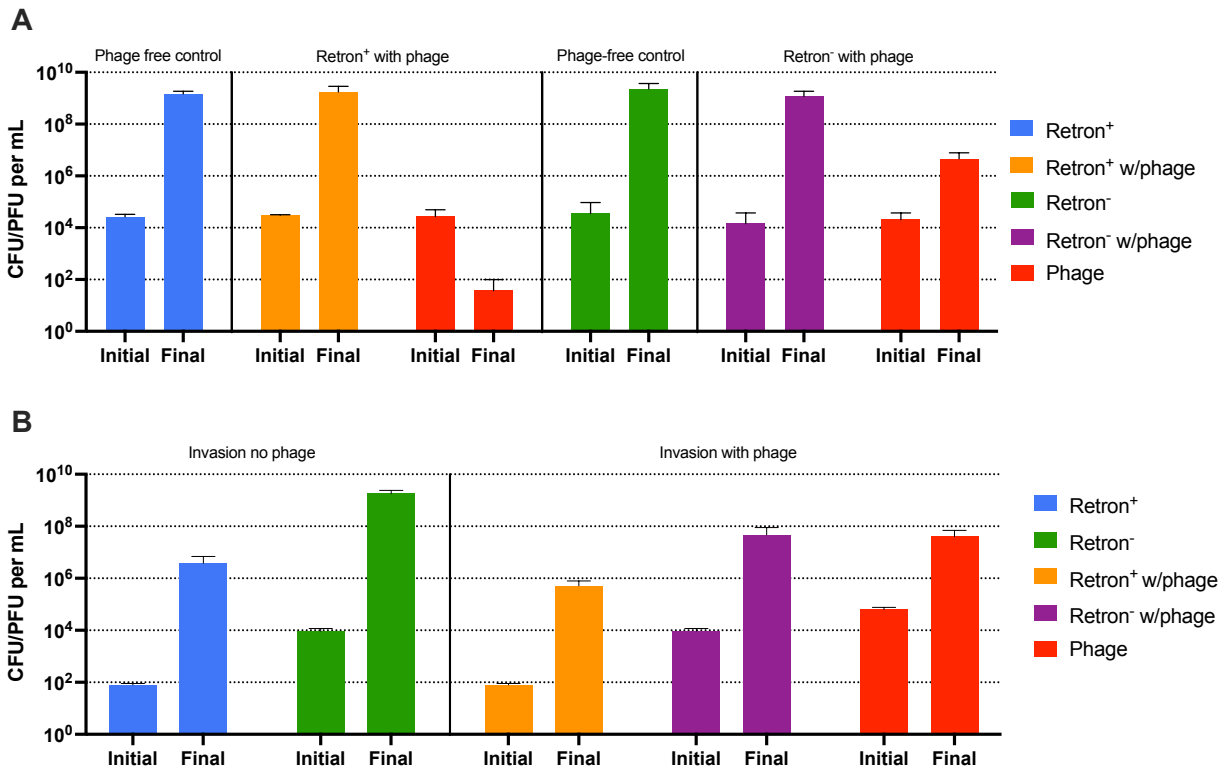


Figure 3. Experimental results of changes in the densities of bacteria and phage at 0 (Initial) and 24 (Final) hours. Retron$^+$ bacteria in the absence of phage (blue) and in the presence of λ^{VIR} (orange), a retron$^-$ population of bacteria in the absence (green) of phage (red) and in the presence (purple) of phage. The data are the means and standard errors of the phage and bacterial densities of three independent replicas. **A-** Retron$^+$ and retron$^-$ populations in the absence and presence of the lytic phage λ^{VIR}. **B-** Invasion experiments. A co-culture of a low density of retron$^+$ bacteria and a high density of retron$^-$ bacteria in the absence and presence of λ^{VIR}.

Considering the presence of a dominant resistant bacterial population, we performed computer simulations with the model presented in Figure 1, but now we allowed for the generation of a λ^{VIR} resistant retron$^+$ population (E_r) and a λ^{VIR} resistant retron$^-$ (N_r) bacterial population. As noted in Chaudhry et al (14), there is a high rate of generation of a λ^{VIR} resistant *E. coli*, suggesting transition rates, μ_{er}, μ_{nr}, μ_{re}, and μ_{rn} of 10^{-5} per cell per hour. With these rates, both the phage and resistant bacteria ascend (Figure 4). This parallels our observation in the previous experiments with λ^{VIR} and a sensitive *E. coli* (Figure 3C). Said another way, if we allow for phage resistant mutants to be generated in our model, the retron$^+$ population can increase in density (Figure 4B, hashed orange), but the dominant population will still be phage-resistant retron$^-$ cells (Figure 4B, hashed purple).

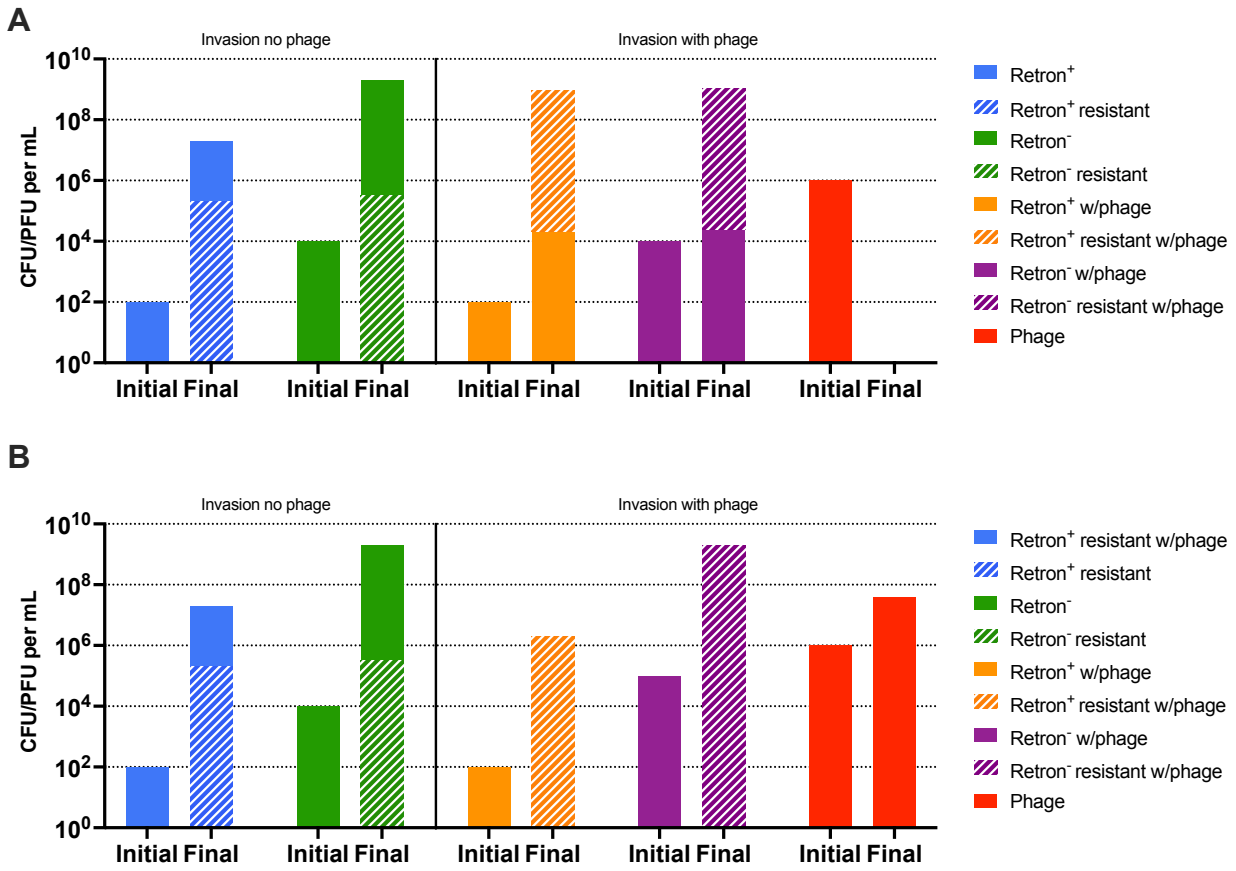
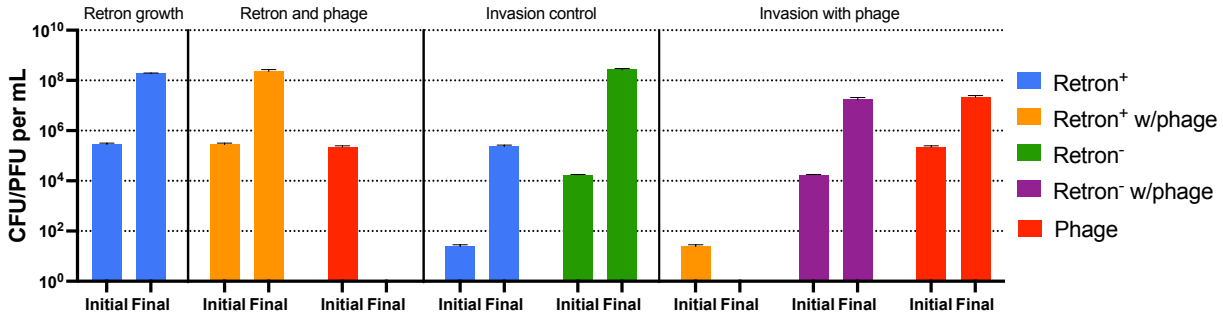


Figure 4. Computer simulations with envelope resistance. The simulation conditions are similar to those in Figure 2C but allow for the generation and selection of resistance, $\mu_{er} = \mu_{re} = \mu_{nr} = \mu_{rn} = 10^{-5}$ per cell per hour. Changes in the densities of bacteria and phage at time 0 (Initial) and 24 hours (Final) for two invasion conditions. Shown in the left side of this figure, are the densities of retron$^+$ (blue) and retron$^-$ (green) bacteria in the absence of phage. On the right side are the densities of retron$^+$ (orange) and retron$^-$ bacteria (purple) in the presence of phage (red). The densities of phage resistant mutants are noted by bars with white hashing over the same bar of the sensitive population. The minor population in all cases is overlayed to the greater. **A-** Simulations with a completely effective retron-mediated abi system (q=1.00). **B-** Simulations with a less-than completely effective retron-mediated abi system (q=0.95).

181 Retron-mediated abortive infection in a physically structured environment
182
183 To explore the contribution of physical structure similar to those depicted in Figure 3B in soft
184 agar, using the methods similar to those employed in (12). As observed in liquid culture, in the
185 physically structured habitat of soft agar, Ec48 protects the bacterial population from being killed
186 off by the phage and prevents the replication of the phage (Figure 5). The retron-lacking
187 population grows to maximum density and the phage are lost. As observed in liquid culture
188 (Figure 3B), the retron$^+$ population is not favored by phage-mediated selection. Also, as observed
189 in liquid culture, all of the λ^{VIR} exposed bacteria recovered at 24 hours were resistant to the phage
190 (Table S3).



191
192 **Figure 5**. Experimental replicates of the results presented in Figure 3, but in a structured
193 environment, presented as means and standard errors from three independent replicas. Shown
194 are the densities of retron$^+$ (blue) and retron$^-$ (green) bacteria in the absence of phage, and the
195 densities of retron$^+$ (orange) and retron$^-$ bacteria (purple) in the presence of phage (red).

196 **Discussion**

197 Our mass action (liquid culture) models predict that a retron-mediated abortive infection system
198 can prevent the populations of bacteria coding for them from being invaded by lytic phages, but
199 only if the retron-mediated abi is nearly completely effective and when there are no other
200 bacterial populations that can support the replication of the phage. The results of our
201 experiments, like those of Millman and colleagues (10) with the retron-encoding abortive
202 infection system in *E. coli,* Ec48, and the phage λ^{VIR}, are consistent with these predictions. In
203 addition to this protection result occurring in liquid culture, our experiments demonstrate that
204 the protection against lytic phages happens in physically structured populations of bacteria
205 maintained in soft agar. Our model also predicts if retron-mediated abortive infection is less than
206 98% effective, with more than 2% of infections being lytic and producing phage, or when there
207 are retron$^-$ populations that can support the replication of the phage, retrons will not be able to
208 protect a population from predation by lytic phages. We were unable to formally test this < 98%
209 efficacy hypothesis experimentally, because our experimental results show that the retron-
210 mediated abortive infection is overshadowed by selection for resistance to the phage. However,
211 since our retron$^+$ population was capable of eliminating the phage population when alone, we
212 interpret this to suggest that the efficacy of the Ec48 abortive infection system is over 98%. Even
213 though this retron-mediated abortive infection system is highly effective, when the bacteria are

214 capable of generating envelope resistant mutants, retron$^+$ or retron$^-$ resistant mutants ascend to
215 dominate the bacterial populations.
216
217 Our model predicts that even if a retron-mediated abi defense system is 100% effective in
218 preventing lytic phage replication, and there is an abundance of phage, the retron$^+$ population
219 will not be able to evolve by abortive infection alone. Stated another way, retron$^+$ bacteria will
220 not be able to become established in a population of retron$^-$ bacteria of similar fitness. Our
221 experiments testing this hypothesis were consistent with this prediction. In liquid culture, the
222 retron-expressing populations were unable to become established in populations dominated by
223 retrons$^-$ competitors. In these experiments, the *E. coli* population surviving this encounter with
224 λ^{VIR}, was dominated by retron-lacking λ^{VIR} resistant mutants.
225
226 Our failure to see the ascent of retron$^+$ bacteria in soft agar is inconsistent with the prediction of
227 the agent-based models of Fukuyo and colleagues (12) and Bergruber and colleagues (13) which
228 demonstrated that in physically structured communities, there are conditions where bacteria
229 with abi systems can invade and become established in populations dominated by abi$^-$ cells
230 sensitive to the co-existing phage. By adding λ phage bearing a DNA methyltransferase Fukuyo
231 and colleagues (12) constructed an *E. coli* with an abi system. Upon infections with a clear mutant
232 of the phage lambda, λ^{CL} (which cannot form lysogens) cells with this abi construct die and the
233 infecting phage is lost. In the physically structured habitat of soft agar (12, 15), in the presence
234 of λ^{CL} this abi-encoding *E. coli* has an advantage over *E. coli* without this abi system, but not in a
235 habitat without this structure. Similar results were obtained by Berngruber and colleagues (13)
236 with a *lit* mutant *E. coli* which, upon infection with the lytic phage T6, dies and aborts the
237 infection. In their experiments with *E. coli* growing as colonies in structed environments,
238 depending on the number and size of the colonies, bacteria with their *lit* abi system were
239 substantially more fit than the competing population of abi$^-$ *E. coli*. In neither of these studies,
240 was the abortive infection able to evolve in liquid culture. Contrary to these two results, with the
241 retron-mediated abi λ^{VIR} system used in this study, the retron-encoding population was unable
242 to evolve in the physically structured habitat of soft agar.
243
244 We end this report on a somewhat philosophical note. It is commonly assumed that the
245 phenotype observed is the object of natural selection. For example, resistance generated by
246 modifications of the receptor sites to which phage adsorb evolves through selection mediated by
247 phage. That selection can be easily demonstrated by exposing sensitive bacteria to phage. There
248 is, however, another side to this. Clearly, the receptor sites to which the phage adsorb did not
249 evolve to adsorb phage, no more than CRISPR and retrons evolved to enable molecular biologists
250 to do genome editing. Throughout this investigation we, and almost all of the abortive infection
251 articles cited, implicitly or explicitly assert that abi evolved in response to selection mediated by
252 phage. Could it be that retrons and other abi systems evolved and are maintained by selection
253 for factors other than as defenses against phage infection?

Materials and Methods

The Mathematical Model

In Figure 1, we illustrate our model of the population dynamics of lytic phage and bacteria with and without a retron-mediated abortive infection system and envelope resistance. There is a single population of phage, P, particles per ml and four bacterial populations of bacteria, E, E_r, N, and N_r cells per ml. The phage sensitive retron population, E, has a functional abi system. Though it also has a function abi system, the E_r population is refractory to the phage. The N and N_r populations are retron negative, retron$^-$, that are, respectively sensitive and resistant to the phage. When a phage infects a bacterium of state E, there is a probability q ($0 \leq q \leq 1$), that the bacteria will die and the infecting phage will be lost. The N population and 1-q of the E population support the replication of the phage while E_r and N_r are refractory to the phage.

The bacteria grow at maximum rates, v_e, v_{er}, v_n, and v_{nr}, per cell per hour, for E, E_r, N and N_r, respectively with the net rate of growth being equal to the product of maximum growth rate, v_{max} and the concentration of a limiting resource, r μg/ml, $v_{max}*\psi(R)$ (16), Supplemental Eq (1). The parameter k, the Monod constant, is the concentration of the resource, at which the net growth rate of the bacteria is half its maximum value. By mutation or other processes, the bacteria change states, $E \rightarrow E_r$ and $E_r \rightarrow E$, at rates μ_{er} and μ_{re}, per cell per hour, and $N \rightarrow N_r$ and $N_r \rightarrow N$ at rates μ_{nr} and μ_{rn}.

The limiting resource is consumed at a rate equal to the product of $\psi(R)$, a conversion efficiency parameter, e μg/cell (17) and the sum of products of the maximum growth rates of the bacteria and their densities. We assume phage infection is a mass action process that occurs at a rate equal to the product of the density of bacteria and phage and a rate constants of phage infection, δ_e and δ_n (ml·cells/hour) for infections of E and N, respectively (18). Infections of N by P produce β_n phage particles, and the (1-q) of the infections of E by P that do not abort, produce β_e phage particles. To account for the decline in physiological state as the bacteria approach stationary phase, R=0, we assume phage infection and mutation rates decline at a rate proportional to Eq.1. The lag before the start of bacterial growth and latent period of phage infection are not considered in this model or the numerical solution employed to analyze its properties.

The changes in the densities of bacteria and phage in this model are expressed as the series of coupled differential equations presented in the supplemental material.

Numerical solutions – computer simulations.

To analyze the properties of this model we use Berkeley Madonna to solve the differential equations (Supplemental Equations (1) – (7)). The growth rate and phage infections parameters used for these simulations are those estimated for *E. coli* and λ^{VIR}. Copies of this program are available at www.eclf.net.

Growth media and strains

Bacterial cultures were grown at 37 °C in MMB broth (LB broth (244620, Difco) supplemented with 0.1 mM $MnCl_2$ and 5 mM $MgCl_2$). The *E. coli* strain containing the Ec48 retron plasmid was obtained from Rotem Sorek. The sensitive *E. coli* used for controls was *E. coli* C marked with streptomycin resistance, and the Ec48 was marked with ampicillin resistance to differentiate in

294 the invasion experiments. The λ^{VIR} phage lysates were prepared from single plaques at 37 °C in
295 LB medium alongside *E. coli* C. Chloroform was added to the lysates and the lysates were
296 centrifuged to remove any remaining bacterial cells and debris. The λ^{VIR} strain used in these
297 experiments was obtained from Sylvain Moineau.

298 **Sampling bacterial and phage densities**

299 Bacteria and phage densities were estimated by serial dilution in 0.85% saline followed by plating.
300 The total density of bacteria was estimated on LB hard (1.6%) agar plates. In invasion
301 experiments, diluted samples were placed on LB hard (1.6%) agar plates supplemented with
302 ampicillin (2.5%) or streptomycin (4%) plates to distinguish retron$^+$ and retron$^-$ *E. coli*. To estimate
303 the densities of free phage, chloroform was added to suspensions before serial dilution. These
304 suspensions were plated at various dilutions on lawns made up of 0.1 mL of overnight LB-grown
305 cultures of *E. coli* C (about 5×10^8 cells per mL) and 4 mL of LB soft (0.65%) agar on top of hard
306 (1.6%) LB agar plates.

307 **Resistance Testing with Cross Streaks**

308 Bacteria were tested by streaking in straight lines ten colonies from 24-hour plates across 20 µL
309 of a λ^{VIR} lysate (>10^8 plaque-forming units [pfu]/mL) on LB hard (1.6%) agar plates. Susceptibility
310 to λ^{VIR} was noted as breaks in the lines of growth. Continuous lines were interpreted as evidence
311 for resistance.

312 **Growth Rate Estimations**

313 Growth rates were estimated in a Bioscreen C. 48-hour overnights of each strain to be tested
314 were diluted in MMB broth to an initial density of approximately 10^5 cells per ml. 10 replicas of
315 each strain were loaded into 100-well plates and grown at 37c with shaking for 24 hours taking
316 OD (600nm) measurements every five minutes.

317 **The Liquid culture experiments**

318 Bacterial overnight cultures grown at 37 °C in MMB Broth were serially diluted in 0.85% saline to
319 approximate initial density and 100 µL were added to flasks containing 10 mL MMB. λ^{VIR} lysate
320 (>10^8 pfum/ml) was serially diluted to an MOI of ~1 and 100 µL was added to the appropriate
321 flask. These flasks were sampled for both phage and bacterial initial densities (t = 0 h) and were
322 then grown at 37°C with constant shaking. The flasks were, once again, sampled for phage and
323 bacterial densities (t = 24 h).

324 **Experiments in soft agar cultures**

325 Bacterial cultures grown at 37°C in MMB and λ^{VIR} lysate were serially diluted in 0.85% saline to
326 appropriate initial densities. The final dilutions were sampled for phage and bacterial initial
327 densities and 100 µL of diluted phage and bacteria were added to 4 mL of LB soft (0.65%) agar
328 and poured into small petri dishes which were grown at 37°C. After 24 hours, the agar was placed
329 into a tube containing 6 mL of saline, vortexed and sonicated in a water bath for 1 hour. These
330 tubes were serially diluted and sampled for final phage and bacterial densities.

331

Acknowledgements

We thank Rotem Sorek and his colleagues at the Weitzmann Institute for providing us with the phage and bacteria used in this study. We thank Kylie Burke, Josh Manuel, Jake Fontaine, Thomas O'Rourke, Yixuan Peng, Ingrid McCall and Eduardo Rodriguez-Roman for their comments and reviews of this manuscript. We are grateful to Ingrid McCall and David Goldberg, for advice and help with the experimental work. Funds for this research were provided by a grant from the US National Institutes of General Medical Sciences, R35 GM 136407 (BRL).

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Supplemental Material - The ecological consequences and evolution of retron-mediated suicide as a way to protect *Escherichia coli* from being killed by phage

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Supplemental Equations

$$\boldsymbol{\Psi(R) = \frac{R}{R+K}}$$

SEq. (1)

$$\boldsymbol{\frac{dR}{dt} = -\Psi(R) \cdot e \cdot (v_n \cdot N + v_{nr} \cdot N_r + v_e \cdot E + v_{er} \cdot E_r)}$$

SEq. (2)

$$\boldsymbol{\frac{dN}{dt} = \Psi(R) \cdot \left(v_n \cdot N - \delta_n \cdot N \cdot P + (\mu_{rn} \cdot N_r - \mu_{nr} \cdot N)\right)}$$

SEq. (3)

$$\boldsymbol{\frac{dN_r}{dt} = \Psi(R) \cdot \left(v_{nr} \cdot N_r - (\mu_{rn} \cdot N_r - \mu_{nr} \cdot N)\right)}$$

SEq. (4)

$$\boldsymbol{\frac{dE}{dt} = \Psi(R) \cdot \left(v_e \cdot E - (\mu_{re} \cdot E_r - \mu_{er} \cdot E)\right)}$$

SEq. (5)

$$\boldsymbol{\frac{dE_r}{dt} = \Psi(R) \cdot \left(v_{er} \cdot E_r - (\mu_{re} \cdot E_r - \mu_{er} \cdot E)\right)}$$

SEq. (6)

$$\boldsymbol{\frac{dP}{dt} = \Psi(R) \cdot (\delta_n \cdot \beta_n \cdot P \cdot N \cdot -\delta_e \cdot P \cdot q \cdot E + (1 - q) \cdot \delta_e \cdot P \cdot E \cdot \beta_e)}$$

SEq. (7)

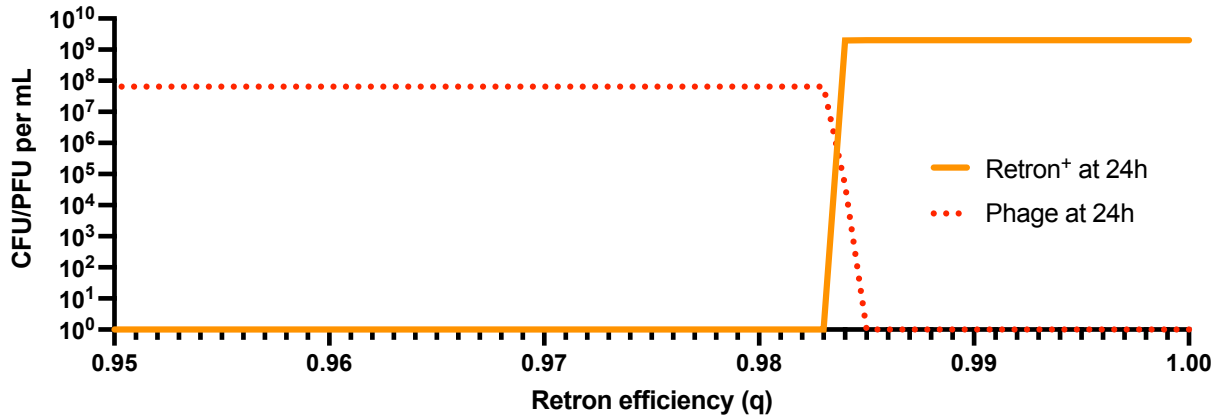

Figure S1. Computer simulation results for the effect of the retron efficiency as values of q. Changes in the densities of a retron⁺ bacterial population in the presence (orange) of phage (red) at 24 hours. Shown are 50 simulations with values of q ranging from 0.95 to 1 with a step size of 0.001. The parameters were the same as in Figure 2.

Table S1. Parameter values used in the simulations.

Parameter	Values (dimensions)	Description	Source (Reference)
v_e, v_{er}, v_n, v_{nr}	$2.00(h^{-1})$	Maximum growth rates	This work (Table S2)
μ_{nr}, μ_{rn}	$1e^{-5}$(per cell per hour)	Transitions $N \rightarrow N_r, N_r \rightarrow N$	(1)
μ_{er}, μ_{re}	$1e^{-5}$(per cell per hour)	Transitions $E \rightarrow E_r, E_r \rightarrow E$	(1)
δ_e, δ_n	$2e^{-7}$(per hour per mL)	Adsorption rates	(1)
β_e, β_n	60 $(PFU \cdot CFU^{-1})$	Burst sizes	(1)
e	$5e^{-7}$(μg per cell)	Conversion Efficiency	(2)
K	1	Monod constant	(2)
q	$0 \leq$ q ≤ 1	Effectiveness of Abi system	This work

410 **Table S2.** Growth rate determination.

Strain (Population)	Symbol	Growth rate (h^{-1})± SE
Escherichia coli Ec48 (E)	v_e	2.27 ± 0.06
Escherichia coli Ec48 resistant (Er)	v_{er}	2.17 ± 0.02
Escherichia coli C (N)	v_n	2.37 ± 0.04
Escherichia coli C resistant (Nr)	v_{nr}	2.27 ± 0.02

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413 **Table S3.** Cross-streak results at 24 hours.

Strain	Plate number	Resistant ratio*
Escherichia coli Ec48	1	1
	2	1
	3	1
	4	1
	5	1
	6	1
	7	1
	8	1
	9	1
	10	1
Escherichia coli MG1655	1	1
	2	1
	3	1
	4	0.9
	5	1
	6	1
	7	1
	8	1
	9	1
	10	1

* Each colony with a minimum number of 7 from a 10^{-7} dilution plate were tested for phage resistance (λ^{vir})and the ratio of
414 resistant/total was calculated.
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