Current Biology 16, 2048–2052, October 24, 2006 ©2006 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2006.08.089

Report

Ecology of Microbial Invasions: Amplification Allows Virus Carriers to Invade More Rapidly When Rare

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Summary

Locally adapted residents present a formidable barrier to invasion [1–3]. One solution for invaders is to kill residents [4]. Here, we explore the comparative ecological dynamics of two distinct microbial mechanisms of killing competitors, via the release of chemicals (e.g., bacteriocins [5]) and via the release of parasites (e.g., temperate phage [6, 7]). We compared the short-term population dynamics of susceptible *E. coli* K12 and isogenic carriers of phage ϕ80 in experimental cultures to that anticipated by mathematical models using independently derived experimental parameters. Whereas phages are a direct burden to their carriers because of probabilistic host lysis, by killing competitor bacteria they can indirectly benefit bacterial kin made immune by carrying isogenic phage. This is similar to previously described bacteriocin-mediated effects. However, unlike chemical killing, viable phage trigger an epidemic among susceptible competitors, which become factories producing more phage. Amplification makes phage carriers able to invade well-mixed susceptibles even faster when rare, whereas chemical killers can only win in a well-mixed environment when sufficiently abundant. We demonstrate that for plausible parameters, the release of chemical toxins is superior as a resident strategy to repel invasions, whereas the release of temperate phage is superior as a strategy of invasion.

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Results and Discussion

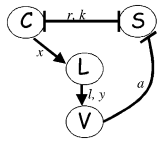
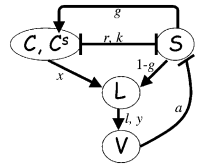
A major mechanism by which microbes kill competitors is through the release of chemical anticompetitor toxins (e.g., antibiotics and bacteriocins), also referred to as allelopathy [4]. Colicins are particularly well-studied bacteriocins produced by the bacteria *Escherichia coli*. Colicinogenic lineages carry genes causing, with a low probability, the explosive suicide of the host and the release of killer proteins or colicins. Nonlysed carriers are immune to the colicin released by their lysed kin, thanks to the specific antidote-coding genes carried along with the colicin genes [5]. Thus the release of colicins can favor carriers over any susceptible residents. Previous experimental and theoretical studies of colicin-mediated bacterial competition have shown that colicin producers can invade in a structured environment (e.g., agar plate) and if unstructured (e.g., shaken flask), then only if sufficiently abundant [8–12]. When the invaders are sufficiently rare, the colicins are too diluted to kill enough residents to compensate for the cost of colicin production.

Escaping the Rarity Threshold in an Unstructured Environment

If the environmental impact of invaders is linear with their numbers, escaping this threshold is theoretically impossible [10–12]. What if, in contrast, the environmental modification the invaders initiate were capable of self-amplification? Rather than accumulating linearly with invader effort, the environmental modification would follow an autocatalytic dynamic. We focus on *E. coli* and some of its phages (viruses of bacteria), which from a bacterial perspective can function as bacteriocins [6, 7] but with the additional capacity of autocatalytic amplification on victims when they are abundant. More specifically, we focus on temperate phage, phage that are transmitted either vertically or horizontally. Infection of susceptible bacteria by temperate phage can result in two possible outcomes. The most common is the lytic cycle (rapid host lysis and production of numerous horizontally transmissible phage particles). However, rarely, the host can be lysogenized by the phage, which persists within its host in a dormant state while allowing the survival of the infected bacteria. This dormant phage is then replicated with the bacterial genome and thus vertically transmitted upon bacterial division. Furthermore, this vertically transmitted carried phage provides immunity to its carrier bacteria against further horizontal infection by this phage [13, 14]. Upon rare induction of the carried phage, phage progeny is released through host lysis.

Through models and experiments, we explore how invasive bacteria-phage complexes can invade populations of phage-susceptible bacteria. We show

Table 1. Summary Comparison of Colicinogenic Plasmids and Temperate Phages

Competition Mediator	Colicinogenic Plasmids	Temperate Phages
Model logic[a] (*C* = carriers, *S* = susceptibles, *L* = latent (pre-burst) cells, *V* = free colicin/phage, *Cs* = new carriers)		
Direct selection on killing agent	No (protein)	Yes (virus)
Transferability from carriers to susceptibles	Weak[b]	Strong
Productivity per lysed cell	High	Low
Amplification on susceptible bacteria	No	Yes
Ecological direct effects	Parasite	Parasite
Ecological indirect effects	Mutualist	Mutualist
Carrier victory	When carriers abundant	When susceptibles abundant
Speed of the carrier victory	Faster with carrier abundance	Faster with susceptible abundance
Ecological "strategy"	Resident	Invader
Unit cost of weapon	Fixed	High initially, then negligible

[a] The model and parameters are introduced in Experimental Procedures and developed in Supplemental Data.
[b] Colicinogenic plasmid can be transferred horizontally ([5] and literature cited), although this property is not considered in our model of chemically mediated competition.

theoretically and experimentally that whereas phage are a direct burden to their bacterial carriers because of probabilistic host lysis, by killing competitor bacteria they indirectly benefit bacterial kin made immune by carrying isogenic phage. We illustrate that unlike colicin producers, bacteria-phage associations can invade well-mixed susceptibles when these associations are arbitrarily rare. Finally, we demonstrate that for plausible conditions, carriage of chemical toxins is superior as a resident strategy, whereas phage carriage is superior as a strategy of invasion.

Model of Phage-Mediated Competition

Our initial population-dynamical model traces the population dynamics of susceptible and phage-carrier populations, mediated by temperate phage. The model logic (related to [7, 9, 15]) is schematized in Table 1 and developed in detail in the Supplemental Data available online.

A stability analysis illustrates that for the specific parameters of our experimental model and for any initial ratio of susceptibles to phage carriers, phage carriers will exclude the susceptible population, given enough time—i.e., there is no rarity threshold to invasion (red lines, Figure 1A). However, deterministic simulations of this system illustrate that the initial ratio of phage carrier to susceptible bacteria can have a significant impact on the transient path to this phage-carrier equilibrium. The phage carriers increase more rapidly when initially rare (Figure 2A). When carriers are rare (and susceptibles abundant), the released phage multiply dramatically on susceptibles, producing a lethal dose of phage leading to a rapid extinction of susceptibles (Figure S1A). Consequently, phage produced by infected susceptibles (V_S) far outnumber phage released by phage-carrier death (V_C, Figure 2B). Similarly, when carrier and susceptible populations are initially equivalent, the number

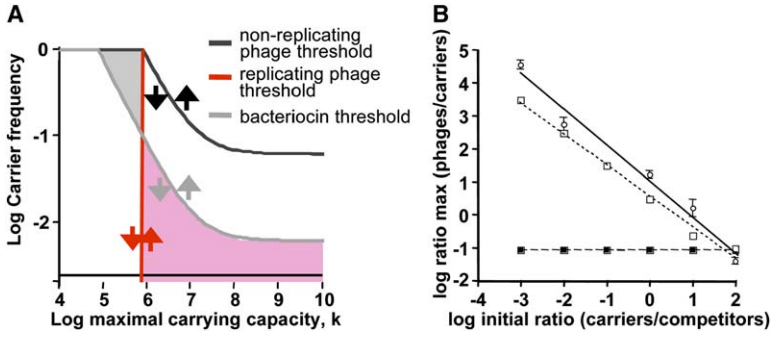


Figure 1. Contrasting Chemical and Viral Competition

(A) Stability analyses of chemical and viral competition. Equilibrium behavior as a function of bacterial density (k) and total carrier frequency ($(C + C^S)/N$). The red line [for $r \gg x$, approximated by $u/a(y - 1)$] outlines virus-carrier stability—to the right of the red line, carriers always win, and to the left of the red line, susceptibles always win (density-dependent competition). The curved lines ($[ak(r - x) + ru]/[aky(r - x)]$) outline chemical-carrier stability. To the above right of these lines, carriers exclude susceptibles; to the below left of these lines, susceptibles exclude carriers (frequency- and density-dependent competition). For large k, carriers exclude susceptibles when carrier frequency > $1/y$. When $k < ru/(a(r - x)(y - 1))$, 100% carriers cannot resist susceptible invasion. The model and parameters are defined in the Experimental Procedures and detailed in the Supplemental Data. Parameter values are the same as in the Experimental Procedures and Figure 2, except for gray line where chemical-weapon burst size has a 10-fold advantage (here, $y = 160$). Gray region (high frequency, moderate density) is where only chemical weapon is efficient. Pink region (low frequency, high density) is where only viral weapon is efficient. Alternate parameterizations are explored in the Supplemental Data.

(B) Amplification as a function of initial susceptible population. We show the maximal ratio of free phage to phage carriers, in experimental and simulation results. Open circles are experimental measures (± SEM) and open squares are simulation results (see Figure 1C); for comparison filled squares are nonreplicating-phage (also known as inefficient bacteriocins) simulation results. Lines represent linear fit to the data.

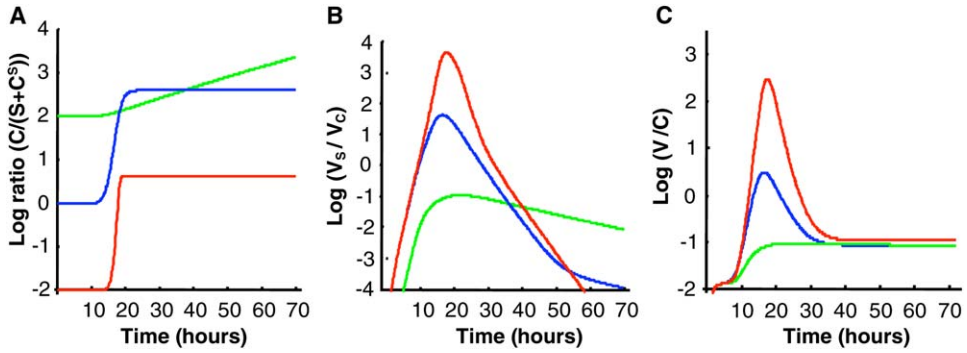


Figure 2. Simulation Competition Results

Temporal dynamics of carrier (*C*) to competitor (susceptible, *S*, plus lysogenized susceptible, C^S) ratio (A) and of susceptible-derived (*Vs*) to carrier-derived (*Vc*) virus ratio (B) and phage (*V*) to carrier (*C*) ratio (C), for differing initial ratios. The initial values were $C = 10^3$ and $S = 10^5$ (red line), $C = S = 10^5$ (blue line), and $C = 10^5$ and $S = 10^3$ (green line). C^5 and *V* have zero initial densities throughout. The model and parameters are defined in the Experimental Procedures and detailed in the Supplemental Data.

of susceptibles is still sufficient for an epidemic to develop ($Vs \gg Vc$, Figure 2B). The resulting peak in phage density is sufficient to rapidly reduce susceptibles to a very low frequency before the loss of susceptibles draws the phage growth to a halt.

Conversely, when susceptibles are initially rare, the released phage population is unable to multiply on the susceptible bacteria because of their low density (Figure S1C). When an initial phage produces on average less than one daughter infection, the maintenance of free phage is due solely to the continued lysis of a fraction of carrier cells. In consequence, we see a near-monotonic growth—driven by phage-carrier lysis—to the equilibrium density of phage, with only a minor contribution from the lytic exploitation of susceptibles ($V_S \ll V_C$, Figure 2B). The variable multiplication of free phage on susceptibles can also be seen in the ratio of free virus to carriers (Figure 2C). In the absence of a sufficient phage peak, the susceptible strain persists over a timescale of many days (Figure S1C).

Note that at equilibrium (e.g., for red and blue lines in Figure 2A, after 24 hr) 100% of the bacteria carry the phage, thus showing a clear gain to the phage (in addition to earlier transient benefits from the peak in free-phage production). However, the phage only partially shares its success with its initial bacterial partner; a fraction of the previously susceptible bacterial lineage persists as phage carriers in a lysogenized and hence immune form (see right diagram, Table 1). Thus at equilibrium the original carrier lineage coexists with the once-susceptible lineage, now in lysogenized form. The gain of the original carriers on the initially susceptible lineage converges on the inverse of the lysogenization rate as the final equilibrium is approached [7]. Of course, if the carriers were to carry multiple phages, as seen in the notoriously invasive *E. coli* 0157:H7 [16], the total gain to the original carriers would be much higher.

Experimental Test

The predictions of the model were then tested experimentally: Isogenic phage-carrier and isogenic susceptible *Escherichia coli* were placed in competition at different initial frequencies in a well-mixed environment (Experimental Procedures). The results of competition between these two lineages are consistent with our qualitative prediction of a faster carrier invasion when initially rare (Figure 2A). When the susceptibles were common (Figures 3A and 3B, blue and red curves), we saw three steps: (1) an initial competitive neutrality; (2) a rapid gain, preceded by a rise in the phage population, of multiple orders of magnitude by the carrier lineage; and (3) competitive neutrality, associated with a decrease of phage population, between the two lineages. This last neutrality reflects the lysogenization of the previously susceptible bacteria (Table S1). In contrast, when the carriers were already dominant and the susceptibles were scarce (Figures 3A and 3B, green curve), we saw no significant change of the ratio of the competing bacterial populations. No increase in phage/carrier ratio was seen, and lysogens represented the expected small minority in the susceptible lineage (Table S1). To illustrate that the gain of phage carriers against common susceptibles is mediated by phage particles and is not a pleiotropic effect of phage carriage on bacterial competitiveness, we placed isogenic phage carriers and phage-resistant bacteria in competition and observed no competitive gain (Figure 3C).

To provide a rigorous experimental test of the difference between autocatalytic and nonautocatalytic invasion assistants, we competed phage carriers with susceptible bacteria that were unable to amplify phage (because of a bacterial gene deletion preventing phage-propagule formation [17]). In this case, the phage operates mechanistically as a vertically transmitted gene coding for an allelopathic chemical. With the autocatalytic component removed, we observed no carrier gain on the timescale of our experiment (Figure 3D). Thus we can conclude that the mortality occurring among amplification-permissive susceptibles (Figure 3A) and attributable to primary infections (phage from carriers) is negligible—in other words, the bacteriocin-like function of this phage is much less efficient than its autocatalytic component in killing competitors.

Contrasting Chemical and Viral Killing of Competitors

To compare more acutely chemical carriers and phage carriers, we modified our model to suppress both amplification in and lysogenization of susceptibles (left diagram, Table 1). Although we focus in our mathematical

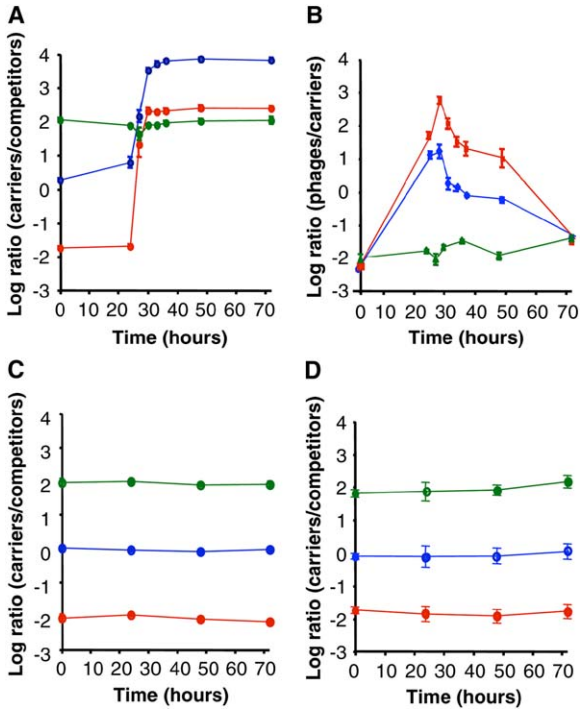


Figure 3. Experimental-Competition Results

(A) Ratio of phage carrier to competitor (susceptible plus lysogenized susceptible) lineages (corresponding to Figure 1A).

(B) Ratio of free phage to carriers (corresponding to Figure 2C).

(C) Ratio of phage carriers to phage resistants. Here, neither the phage carriers nor the phage-resistant competitors carry TonB, a required protein for phage adsorption and infectivity. Note that for these conditions, the cost of carrying a bacteriophage is not detectable in the timescale of our experiments, consistent with the small magnitude of the measured induction rate ($x = 3.7 \times 10^{-3}$ per lysogenized bacteria per hr), and suggesting that further pleiotropic costs are negligible on this timescale.

(D) Normalized ratio of phage carriers to susceptible (but nonamplifying) competitors. The susceptible bacteria lack IhfA, a protein required to produce viable phage progeny. Thus after infection, cells are killed but no new phage particles are released. The ratio is normalized for the *ihfA*$^-$ pleiotropic cost. All data points are the mean of six replicates, three with one coupling of two antibiotic markers and three with the reverse coupling. Populations of susceptibles and lysogenized derivatives of susceptibles were followed by monitoring the antibiotic-resistant population; thus we do not distinguish between them. Bars depict standard errors of the mean (sometimes hidden by the symbols). Color coding distinguishes initial densities of carriers and susceptibles, following Figure 2.

models on the specific kinetics of colicins, our results can be generalized to a range of microbial allelopathic chemicals spanning microcins, bacteriocins, and antibiotics. In Figure 1A (black and gray lines), we illustrate a classical result that the exclusion of susceptibles by chemical carriers can only occur above a given frequency threshold [10–12], defined here in the limit of dense populations as the inverse of the number of lethal doses released by a single cell. Furthermore, we show that this frequency threshold increases as bacteria become scarce, because loss of the chemical agent becomes increasingly important (for more details, see Supplemental Data). Both experimental and model results illustrate how the level of amplification in phage-mediated competition is determined by the availability of susceptibles in the initial population (Figure 1B). When susceptibles are sufficiently rare, the autocatalytic and nonautocatalytic models converge (Figure 1A, red and black lines converge as frequency of susceptibles diminishes) because the phage ceases—as a result of the rarity of susceptibles—to gain any multiplicative advantage, acting purely through its direct killing effect.

However, whereas phage can act essentially as a bacteriocin-style nonmultiplicative killer when their carriers are numerically dominant, they do so in a less efficient manner (Figure 3D) because of their relatively wasteful capacity for replication (e.g., effective colicin "burst size" is far greater [12, 18]). Introducing an advantage to chemical carriers in the number of lethal doses produced on the lysis of a single carrier cell (gray line, Figure 1A) creates a region of parameter space where a chemical carrier could repel rare susceptible invaders, but a phage carrier could not (gray region—high frequency, moderate density—in Figure 1A). However, despite this production advantage for the chemical carrier, the phage carrier continues to have a unique advantage when sufficiently rare (pink region—low frequency, high density—in Figure 1A; Supplemental Data). The extent to which a chemical carrier has an advantage when dominant depends on the quantitave balance of lethal dose and rates of chemical decay and adsorption (Supplemental Data, Figure S4).

The bacteriocin-like properties of temperate phage have begun to receive experimental and theoretical attention [6, 7]. Here, we illustrate that although bacteriocins and temperate phage can provide analogous benefits to their carriers in so far as both can kill competitors, these two mechanisms of bacterial spite [19] display important dynamical differences, limiting their effectiveness in distinct ways. The broader ecological and evolutionary ramifications of these distinctions are wide ranging. We summarize these ecological distinctions while hinting at some of the broader ramifications in Table 1. The cost and benefit of viral versus chemical killing from a carrier perspective depend critically on the abundance of susceptibles in the environment. Whereas the invasion speed of colicin carriers decreases with their initial frequency [12], phage carriers invade more quickly when rare (Figures 2A and 3A). When susceptibles are common (e.g., when carriers are rare and invading a susceptible population), a viral weapon can be the most cost effective because the invaders can co-opt susceptible residents into machines making more of the viral weapon. In contrast, when susceptibles are scarce (e.g., when carriers are dominant and face rare susceptible invaders), a specialized chemical weapon is more efficient because amplification on susceptibles is a negligible contributor to weapon density. Thus we conclude that chemical toxins will be favored in the context of resident defense, and temperate-phage carriage will be favored in the context of invader offense.

Experimental Procedures

Parameter Estimation

The following parameter estimates were made experimentally while bacteria were at carrying capacity (in stationary phase), except for the estimation of the maximum growth rate, $r = 1.1$/hr: carrying capacity (maximum density of susceptibles), $k = 2 \times 10^9$ bacteria/ml; induction or lysis rate, $x = 3.7 \times 10^{-3}$ per infected bacterium per

hr; burst size, $y = 16$ phage per burst; phage mortality rate, $u = 4.4 \times 10^{-3}$/hr; phage adsorption constant, $a = 3.5 \times 10^{-10}$ per bacterium per ml per hr; latency rate, $l = 0.35$/hr; probability of lysogenization, $g = 2.3 \times 10^{-3}$ per infected bacterium. Alternate parameters and parameter estimation methods are presented in the Supplemental Data.

Theoretical Models

The simulations in Figure 2 track the densities of phage carriers (C), susceptibles (S), free-phage (V), latent cells (L), and lysogenized susceptibles, (C^S), according to the following differential equations (parameters defined above, $N = C + S + C^S$):

$$\begin{aligned} dC/dt &= (r(1 - N/k) - x)C \\ dS/dt &= (r(1 - N/k) - aV)S \\ dC^S/dt &= r\left(1 - N/k\right) - x\Big)C^S + gaVS \\ dL/dt &= x\left(C + C^S\right) + (1 - g)aVS - lL \\ dV/dt &= ylL - V(u + aN) \end{aligned} \qquad (1)$$

The stability analyses in Figure 1A explore model 1 together with an alternate chemical model (Table 1), where there is no lysogenization and production of the chemical weapon is linked only to the lysis of carrier cells, i.e., $dL/dt = xC - lL$. All models are introduced and analyzed in more detail in the Supplemental Data. Analyses and simulations were performed in Mathematica 4.0 (Wolfram Research, Illinois, 1994).

Bacterial and Phage Strains

We used *E. coli* MG1655 nalidixic-acid- or streptomycin-resistant variants (MGN and MGS, respectively) that we have lysogenized with a ϕ80-immunity phage (MGNϕ and MGSϕ, respectively). Isogenic-resistant ΔtonB [20] were constructed by the methods described by Datsenko et al. [21]. Isogenic nonamplificative Ihf$^-$ strains were constructed by P1 transduction from a Δ*ihfA* strain [17] with associated resistance to kanamycin.

Experimental Competitions

Ten milliliters of Luria-Bertani (LB) liquid medium were inoculated by a different ratio of MGSϕ/MGN or MGNϕ/MGS (but always with 10^6 total bacteria) and then incubated at 37°C with shaking. At 24 and 48 hr of growth, cultures were diluted 1000 times. At 24, 27, 30, 33, 36, 48 and 72 hr, 500 μl samples were removed to follow bacterial and phage populations. Appropriate dilutions were plated on nalidixic acid and streptomycin LB plates to follow the competing populations. To follow phage populations, we centrifuged samples and poured a mix of supernatant dilutions and 100 μl MG1655 (sensitive bacteria) on overnight culture with 3 ml of Top Agar (7 g/liter). Identical methods were used for competition between ΔtonB noncarriers and carriers of the lysogenic phage, as for the competition between Δ*ihfA* noncarriers and carriers of the lysogenic phage.

Supplemental Data

Supplemental Data include Experimental Procedures, four figures, and one table and are available with this article online at: http://www.current-biology.com/cgi/content/full/16/20/2048/DC1/.

Acknowledgments

We are grateful to P. Berche, J.J. Bull, C. Combes, M. Fons, A. Gomez, B. Grenfell, M. Hochberg, L. Keller, K. Laland, B. Levin, M. Loreau, R. Moxon, J. Odling-Smee, S. Reader, and the group of M. Radman for their comments on the different versions of the manuscript. S.P.B. thanks the European Union, Wellcome Trust, and Human Frontier Science Program (HFSP) for financial support. L.L.C., M.D.P., and F.T. thank the Actions Concertées Incitatives (ACI) and PhD program from the French Ministry of Research, the Institut National de la Santé et de la Recherche Médicale (INSERM), and the Bettencourt Schueller Foundation for financial support.

Received: April 26, 2006
Revised: August 22, 2006
Accepted: August 23, 2006
Published: October 23, 2006

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