

Highlight

Toxins go viral: phage-encoded lysis releases group B colicins**Katrina van Raay and Benjamin Kerr****Department of Biology and BEACON Center for the Study of Evolution in Action, University of Washington, Seattle, WA 98195, USA.*

The natural world is filled with instances of competition between organisms. One extreme form of competition—*allelopathy*—involves the production of chemicals that harm or kill competitors. Such chemical warfare is ubiquitous in the microbial world. Nearly every bacterial lineage studied to date contains strains that produce proteinaceous antimicrobials called bacteriocins (Riley and Wertz, 2002a). The best-understood types are the *colicins*, produced by and active against *Escherichia coli* and its relatives (Cascales, 2007). For one group of these colicins (group A), *allelopathy* is encoded in a three-gene operon housed on a plasmid. One gene encodes the toxin (colicin), a second gene encodes an immunity protein, which binds and neutralizes the toxin, and a third gene encodes a lysis protein. Under stressful conditions, a fraction of plasmid-bearing (colicinogenic) cells turn on the operon, produce toxin, and lyse, releasing the toxin to the external environment. Cells that lack the plasmid die upon exposure to the toxin. In cells that contain the plasmid, the immunity protein, which is constitutively expressed, prevents cell death (Riley and Wertz, 2002b; Cascales *et al.*, 2007). Thus, active producers kill sensitive competitors, allowing their latent immune clones to utilize liberated resources (Riley and Gordon, 1999; Kerr, 2007).

However, there exists another group of colicin systems (group B) that has only the toxin and immunity genes and appears to lack the lysis gene (van der Wal *et al.*, 1995; Cascales *et al.*, 2007). It is a mystery how these colicins get out of their host cells. In an exciting new study that appears in this issue, Nedialkova *et al.* (2016) show that *prophages* may offer a route of escape for the group B colicin Collb. Prophages are bacterial viruses that have been incorpo-

rated into the bacterium's genome (bacteria carrying prophages are called lysogens) (Campbell, 2003). Prophages are common in bacteria, comprising up to 20% of bacterial genomes (Bondy-Denomy and Davidson, 2014). Similar to colicin plasmids, prophages can be vertically transmitted within bacterial lineages without induction. However, under certain circumstances, the prophage can be induced, resulting in the production of progeny phage and lysis of the host cell. Interestingly, some of the same conditions (those activating the SOS response) that induce prophages also induce colicins. In this way, prophage systems may offer an escape route for group B colicins.

Nedialkova *et al.* demonstrate the plausibility of this scenario through both additive and subtractive engineering. Specifically, the authors transformed the Collb plasmid into a prophage-free *E. coli* strain. This transformant produced colicins, but these toxins were not released from the producing cell. The authors then transduced the Collb *E. coli* with a temperate phage, 933W, and observed the release of the colicins. The focal bacterium of this study, *Salmonella enterica* serovar *typhimurium*, contains four prophages and the Collb plasmid. With the prophages intact, colicin is released. However, when one of the phages, ST64B, is deleted, the release of colicin is dramatically reduced.

For both *E. coli* and *S. enterica*, the lysis proteins of the phage appear to be critical for colicin release. Transduction with a lysis-deficient 933W phage is insufficient for colicin release from *E. coli*. Similarly, the loss of the lysis protein of ST64B can reduce colicin release from *S. enterica*. Using reporter assays, the authors additionally show that the timing of toxin production precedes the timing of phage-encoded lysis. Collectively, their experiments provide evidence that phage systems can complement the release deficiency of group B colicins.

Further, the authors demonstrate that phage-mediated release is imperative for successful allelopathic effect in a community context: colicinogenic cells harbouring the prophage dramatically outcompete colicin-sensitive strains in co-cultures. However, colicinogenic cells without the prophage (or lacking phage lysis genes) have reduced

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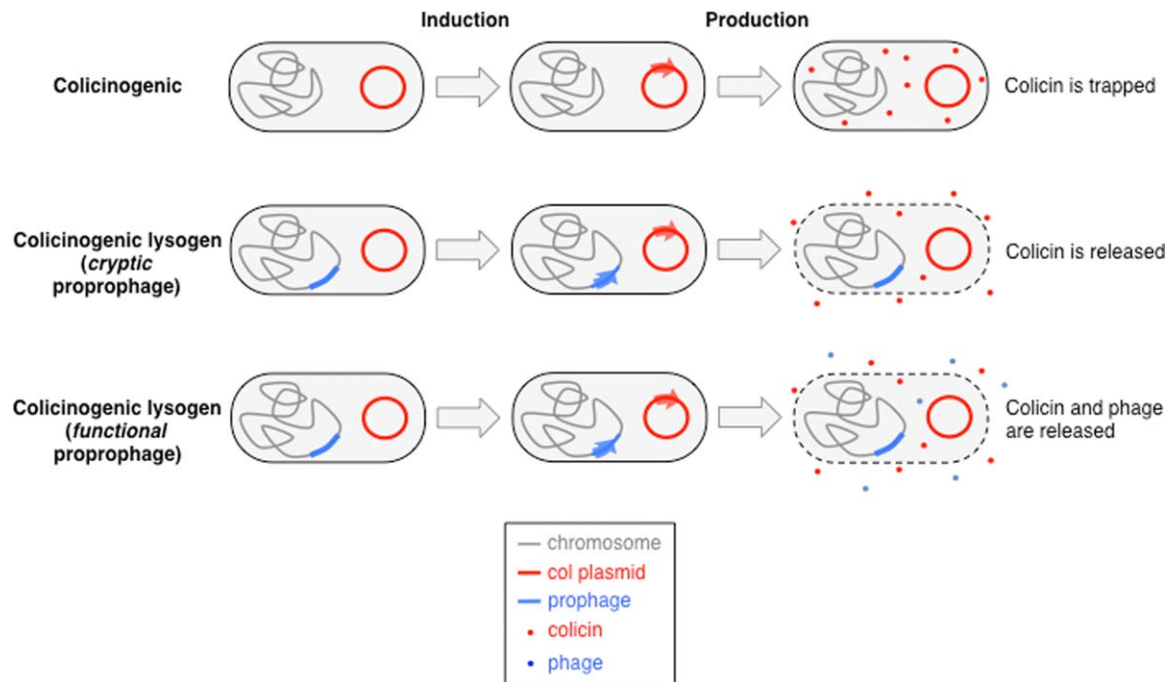


Fig. 1. Schematic representation of the combination of phage and bacteriocin systems. **Top.** *Colicinogenic bacterium* holds a group B colicin plasmid but lacks a lysis gene. Colicin production is induced by the SOS response, but any colicins produced are trapped within the cell. **Middle.** *Colicinogenic lysogen bacterium with cryptic prophage* contains both a group B colicin plasmid and a prophage that cannot produce virions. Even though this cell is not able to produce infectious virions upon induction, the lysis gene of the prophage is functional. The timing of lysis expression is such that the cell lyses after colicins have been produced, releasing colicins into the environment. **Bottom.** *Colicinogenic lysogen bacterium with functional prophage* containing both a group B colicin plasmid and a virion-producing prophage. Under induction, colicins and phage are produced, and released into the environment after cell lysis.

competitive ability. Thus, the combination of two killing systems (phage and colicin) can have ecological consequences with profound effects on community structure.

Once the colicin and prophage systems come together in a cell, why is the union successful? By modifying a mathematical model of Brown *et al.* (2006), we explore the consequences of bringing prophage and group B colicin systems together in a bacterial community (Box 1 and Fig. 1). Our model shows that when the lysogen produces infectious virions, the colicin system can piggyback to high frequency along with the prophage system (Fig. B1A). In this scenario, vulnerable cells could be hit with both colicins and phage. Thus, if these two killing systems can come together into the same cell, there is potential for this “colicinogenic lysogen” to drive itself to dominance in the community.

However, prophage can become dysfunctional in bacteria through selection or neutral drift, such that the lysogen no longer encodes for infectious virions; such prophages are referred to as “cryptic” (Bobay *et al.*, 2014; Casjens, 2003). In the system described by Nedialkova *et al.*, the prophage ST64B is one such cryptic prophage, where a single frame shift mutation prevents infectious virions from being produced but maintains the lysis gene (Figuroa-Bossi and Bossi, 2004). How does a strain containing both

a cryptic prophage (with a functional lysis gene) and a colicin system fare in a bacterial community?

It turns out that crypsis is the Achilles’ heel of the colicinogenic lysogen, which is now dominated by strains that produce colicins but have no prophage (Fig. B1B). The colicinogenic-only strain (Fig. 1, top panel) benefits by having immunity to the colicin, but does not incur the cost of killing itself. Here, the colicinogenic strain is a kind of ‘cheater’, taking advantage of the bacteriocins released by the colicinogenic lysogen with a cryptic prophage (Fig. 1, middle panel) while avoiding the cost of releasing these ‘public goods’. This cheater does not allow the colicinogenic lysogen to rise to high frequency. This result motivates a follow-up question: are there circumstances where a colicinogenic lysogen with a cryptic prophage can dominate strains that are only colicinogenic?

One possibility is that the benefits conferred by carrying the prophage outweigh the cost associated with suicide (particularly if suicide is infrequent). Cryptic prophages can provide other benefits to their hosts, including immunity to infection by other phage (coinfection), resistance to antibiotics, and genes for virulence factors (Brüssow *et al.*, 2004; Tucker, 2004; Wang *et al.*, 2010; Fortier and Sekulovic, 2013). However, if the prophages themselves are beneficial, a different kind of cheater then becomes

possible: a strain that drops the phage lysis gene but retains the advantageous components of the prophage.

Both kinds of cheaters may be kept in check by another feature of microbial life: spatially limited dispersal and interaction. While the model in the Box assumes a well-mixed community (as in a well-shaken flask), natural communities of microbes are often structured spatially (e.g., in biofilms). Given such spatial structure, only a bacterium that releases bacteriocin allows its clones to expand into the territory held by sensitive neighbors (Chao and Levin, 1981; Kerr, 2007). Due to spatially restricted dispersal of cells and limited diffusion of toxins, the potential for a cheater to free ride off the releasing strain is diminished. Thus, spatial structure may account for the rise of bacteria holding both a colicin plasmid and a cryptic prophage encoding cell lysis, as in the system described by Nedialkova *et al.*

Given the ubiquity of both prophages and bacteriocins in bacterial populations, it seems likely that these (and other) killing systems come together with reasonable frequency. Such a union could complement one or multi-

ple dysfunctional partners or synergistically magnify the effects of both. Indeed there are cases where group A colicins have come together with group B colicins in a single cell (Gordon and O'Brien, 2006). Similarly, multiple prophage systems might join forces within a cell. The focal strain of *S. enterica* in this study has, in addition to the cryptic prophage ST64B, three functional prophages. In a population of bacteria, the timing of virion release of two of these functional prophages is offset such that there are two waves of virion release: one subset of lysogens releases virions after the other (Bossi *et al.* 2003). This allows virions from the second prophage to infect and kill competitor cells that have been lysogenized by virions from the first, resulting in a second chance to decrease the population of competing cells. Here, two prophages together enhance the ability of the strain to kill competitors.

In their study, Nedialkova *et al.* show that a dysfunctional prophage can complement a dysfunctional colicin in a bacterium and result in a more effective killing system. The

Box: Mathematical model to explore community dynamics of colicin and phage systems

Nedialkova *et al.* address community-level consequences of the association of phage and bacteriocin systems through pairwise competition experiments. In this box, we further explore potential dynamics for this community by adjusting and extending a mathematical model developed by Brown *et al.* 2006. Using a system of differential equations, we track the density of sensitive bacteria (S), lysogens (L), colicinogenic bacteria (C), colicinogenic lysogens (X), free phage (P), and free bacteriocin (B):

$$\begin{aligned}\dot{S} &= r_S \left(1 - \frac{N}{K}\right) S - a_P P S - a_B B S \\ \dot{L} &= \left\{ r_L \left(1 - \frac{N}{K}\right) - p \right\} L + l a_P P S - a_B B L \\ \dot{C} &= \left\{ r_C \left(1 - \frac{N}{K}\right) - \alpha p \right\} C - a_P P C \\ \dot{X} &= \left\{ r_X \left(1 - \frac{N}{K}\right) - p \right\} X + l a_P P C \\ \dot{P} &= (y_P(1-l) - 1) a_P P (S+C) + y_P p (L+X) - u_P P \\ \dot{B} &= y_B p (\alpha C + X) - u_B B\end{aligned}$$

The growth rate of bacterial strain i is given by r_i . The total density of bacteria is N . The carrying capacity of the system is K . The rate of attack of phage and bacteriocin to vulnerable cells is a_P and a_B , respectively. The probability that a vulnerable cell infected with a phage becomes a lysogen is l . The rate that a lysogen produces and releases phage is p . The factor α measures the reduction in bacteriocin release for a colicinogenic strain; when there is no lysis gene for such a strain, $\alpha = 0$. The yield of phage and bacteriocin from a cell producing and releasing these particles is y_P and y_B , respectively. The rate of decay of phage and bacteriocin is u_P and u_B , respectively. We use the parameter values from Brown *et al.* (2006) with the following modifications: $r_L = 1.0$, $r_C = 1.0$, $r_X = 0.9$, $y_B = 1000$, $u_B = 4.4 \times 10^{-2}$, and $\alpha = 0$. Similar to the results of Nedialkova *et al.*, when the colicinogenic strain does not release bacteriocins, it does not dominate the sensitive strain in a two-strain community. However, if the bacteriocin and phage systems are combined, the colicinogenic lysogen can displace all other bacterial strains (Fig. B1A). It is important to note that lysogens produce infectious phage in this model. Due to production of phage that either kill or convert the sensitive bacteria, the lysogen can exclude the sensitive strain when these two strains are alone. When the two killing systems come together, the bacteriocin system effectively "piggybacks" on the success of the phage system and the colicinogenic lysogen dominates despite slower growth.

In the system studied by Nedialkova *et al.*, the lysogen does not produce infectious ST64B phage. Thus, we reconsider our system without the free phage component. With this change, sensitive cells outcompete colicinogenic cells when the two strains are alone; and sensitive cells similarly displace lysogens in isolation. However, when all four bacterial strains are present, the (formerly ineffective) colicinogenic strain ends up dominating (Fig. B1B). The colicinogenic strain can be seen as a kind of cheater, failing to release costly bacteriocins, but benefiting from the colicinogenic lysogen that produces and releases the bacteriocin that kill the sensitive and lysogenic strains. An important point here is that in a well-mixed system, the colicinogenic lysogen strain the authors describe would be susceptible to displacement by a release-deficient strain.

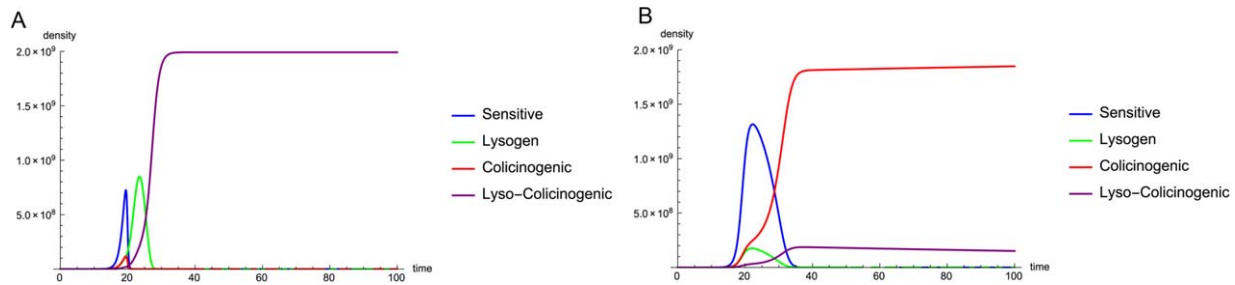


Fig. B1. Community dynamics predicted by a simple mathematical model. (A) When lysogens produce infectious phage, the colicinogenic lysogen is predicted to dominate the community. **(B)** When lysogens do not produce infectious phage, the community is dominated by the colicinogenic strain, which benefits from the bacteriocin released by the colicinogenic lysogen, but avoids the costs (including suicide) associated with the colicinogenic lysogen.

strain additionally benefits by housing productive prophages that increase its virulence and allelopathic impact. What other fascinating unions remain to be discovered? What are their effects at the cell, population, and community level? Certainly, there are many exciting avenues for exploration, such as investigating the mechanisms behind different killing systems, their interactions with each other, and their ecological and evolutionary consequences.

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