

Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage

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Abstract

A major goal of community ecology is to link biological processes at lower scales with community patterns. Microbial communities are especially powerful model systems for making these links. In this article, we review recent studies of laboratory communities of bacteria and bacteriophage (viruses that infect bacteria). We focus on the ecology and evolution of bacteriophage-resistance as a case study demonstrating the relationship between specific genes, individual interactions, population dynamics, community structure, and evolutionary change. In laboratory communities of bacteria and bacteriophage, bacteria rapidly evolve resistance to bacteriophage infection. Different resistance mutations produce distinct resistance phenotypes, differing, for example, in whether resistance is partial or complete, in the magnitude of the physiological cost associated with resistance, and in whether the mutation can be countered by a host-range mutation in the bacteriophage. These differences determine whether a mutant can invade, the effect its invasion has on the population dynamics of sensitive bacteria and phage, and the resulting structure of the community. All of these effects, in turn, govern the community's response to environmental change and its subsequent evolution.

Keywords

Bacteria, bacteriophage, community structure, evolution, host range, mutations, resistance.

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INTRODUCTION

Biological communities are extraordinarily complex, among the most complex entities studied by scientists. This complexity makes understanding communities extremely difficult, and requires that a number of different approaches be used. One particularly fruitful approach has been to strip away much of this complexity and ask fundamental questions of very simple communities. This approach, called the “community module” approach, has been used primarily by theoretical ecologists to identify general processes and qualitative features of communities (e.g. Abrams 1993; Holt *et al.* 1994; Begon & Bowers 1995; McCann *et al.* 1998). Using this approach, theoretical ecologists have, for example, demonstrated the potential importance of indirect interactions and chaotic dynamics in biological communities, two subjects that later became central themes in community ecology (Holt 1995).

This approach has a long history among experimental ecologists as well (e.g. Gause 1934), and growing

numbers of experimental ecologists are turning to simple experimental communities to provide insights into community ecology (e.g. Nakajima & Kurihara 1994; Harrison 1995; Lawler 1995; McGrady-Steed *et al.* 1997; Bohannan & Lenski 1999). Microbial model communities are ideal for such studies (Drake *et al.* 1996). With microbial communities it is possible to study aspects of community ecology that would not be possible to study in other systems, aspects such as the evolution of communities in different environments and the multilayered feedback between genes, individuals, populations and the community. In this article, we review recent studies of simple laboratory communities of bacteria and bacteriophage. From these studies a picture emerges of how subtle genetic differences among individuals can affect the ecological interactions among populations, how these population-level affects then change the community properties, how community properties influence evolutionary dynamics, and how evolutionary dynamics feed back into this process by affecting genetic properties of individuals.

Microbial communities have several advantages as model systems for studying ecology and evolution. Most microbes that are routinely grown in the laboratory have short generation times and are easily maintained in large populations, allowing rapid evolutionary change and the opportunity to study communities on both ecological and evolutionary time scales. Moreover, both environmental and genetic variables can be experimentally manipulated with relative ease in laboratory communities of microbes. Many microbes can be stored indefinitely in a non-evolving state (e.g. frozen at -80°C), which allows direct comparison between ancestral and evolved forms. Microbial populations can be maintained under simple, defined conditions that closely approximate the assumptions of many theoretical models. There is tremendous ecological and genetic diversity among microbial taxa that can be drawn on to assemble model communities. Microbes are widely used as model organisms in other fields, so there exists extensive information on genetic variation and the molecular bases of interactions. Finally, microbes are important in ecosystem processes, industry, agriculture and human health, and studies of microbial communities have direct relevance to these fields.

Laboratory communities of bacteria and bacteriophages have been proposed as model systems (Campbell 1961; Levin & Lenski 1985) and have been successfully used as such by a number of researchers (Paynter & Bungay 1969, 1971; Horne 1970; Chao *et al.* 1977; Levin *et al.* 1977; Levin & Lenski 1983; Lenski & Levin 1985; Lenski 1988c; Schrag & Mittler 1996; Bohannan & Lenski 1997, 1999, 2000; Wichman *et al.* 1999; Crill *et al.* 2000). There are two main groups of bacteriophages: temperate phages (i.e. phages that can lie dormant in bacterial cells after infection) and lytic phages (i.e. phages that invariably kill the host cell following infection and phage reproduction) (Lenski 1988c). Most studies that have used bacteriophage as predators in laboratory communities have used lytic phages. The lytic T-series bacteriophages are voracious consumers of the bacterium *Escherichia coli* and are the most commonly used model predators (Chao *et al.* 1977; Levin *et al.* 1977; Lenski & Levin 1985; Schrag & Mittler 1996; Bohannan & Lenski 1997, 1999, 2000), although virulent (lytic) mutants of phage lambda have also been used (Horne 1970; Schrag & Mittler 1996).

This review will focus on the ecology and evolution of phage-resistance as a case study demonstrating the relationship between individual interactions, population dynamics, community structure, and evolutionary change. We will discuss the conditions under which phage-resistant bacteria evolve and invade laboratory communities, the role of the environment in determining the rate at which this occurs, and the effect of invasion on population stability, community structure and the re-

sponses of communities to environmental change. We will also review studies which demonstrate that subtle differences in the molecular bases of the interactions between bacteria and bacteriophage (e.g. differences in the magnitude of the tradeoff between resistance and competitive ability) determine whether a mutant can invade and what effect its invasion has on population and community properties. Our review will focus on recent studies of communities assembled with phage T4 and *E. coli* B, but we will also discuss communities assembled with other T-phages when such communities differ from those assembled with T4. We will begin with a brief introduction to bacteriophage-bacteria biology.

AN OVERVIEW OF THE BIOLOGY OF PHAGE-BACTERIA INTERACTIONS

The life cycle of a lytic phage begins with its adsorption to the surface of a bacterium (Fig. 1). Adsorption of T-series phage to the bacterial surface occurs through an interaction between the distal end of the phage's tail fibres and a receptor molecule on the cell surface (Goldberg *et al.* 1994). The interaction between tail fibre and receptor molecule is highly specific. For example, phage T4 tail fibres bind only to the glucosyl- α -1,3 terminus of rough lipopolysaccharide (LPS) in *E. coli* B, and only to the OmpC outer membrane protein in *E. coli* K12; phage T2 binds to either the outer membrane protein OmpF or the glucosyl- α -1,3 terminus of rough LPS in *E. coli* B; and phage T7 binds only to the heptose residues in the LPS core (Goldberg *et al.* 1994).

This initial specific binding is weak and reversible. Once this binding has occurred, the phage is thought to "walk" around the bacterial cell until an injection site is reached (Goldberg *et al.* 1994). At the injection site the phage attaches irreversibly to the bacterial cell. The specific phage and bacterial receptors involved in irreversible binding are unknown. Once irreversible binding occurs, the phage DNA is injected into the bacterial cell.

Infection with phage DNA blocks the synthesis of bacterial DNA, RNA and proteins, and it turns *E. coli* into an efficient factory for the production of phage (Goldberg *et al.* 1994). After a latent period during which phage particles are assembled, the bacterial cell is destroyed and the phage progeny are released. The total burst size of the phage (the total number of phage progeny released per bacterial cell) and the latent period can be affected by the nature of the growth media and the physiological state of the bacterial cell (Hadas *et al.* 1997).

Mutant bacteria that are invulnerable to phage infection have been reported for all the T-series phages (Lenski 1988c). Most phage-resistant mutants achieve their

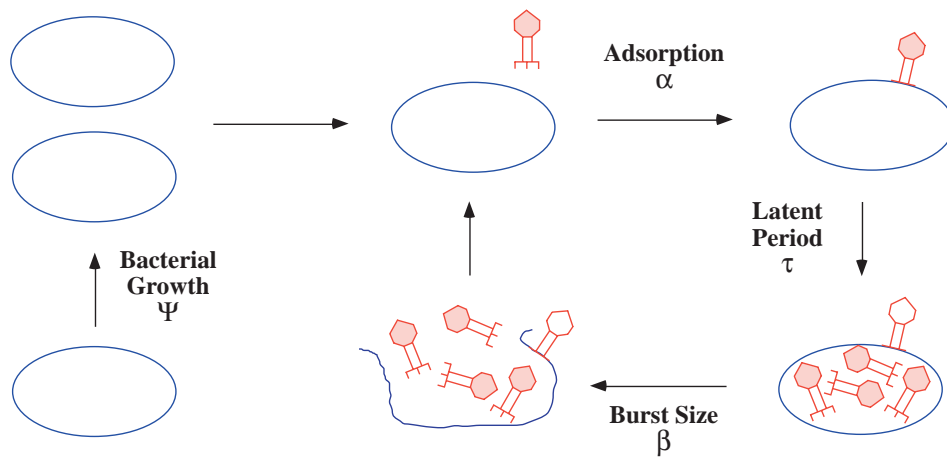


Figure 1 Life cycles and interaction of lytic phage (red) and bacteria (blue).

resistance through the loss or modification of the receptor molecule to which the phage initially binds. These mutations often simultaneously reduce the cell's competitiveness because the receptor molecules are also involved in bacterial metabolism (Lenski 1988a). Despite this cost of resistance, it is commonly observed that phage-resistant mutants quickly evolve and invade most laboratory communities of T-series phages and *E. coli*. In fact, the tradeoff between resistance and competitiveness allows the stable coexistence of sensitive and resistant *E. coli* when phage are present (Lenski 1988c).

Communities of phage and bacteria can be remarkably stable. Coexistence of phage T4 and *E. coli* has been reported for periods as long as 52 weeks (Horne 1970), and in a diversity of laboratory environments including seasonal (i.e. batch culture), continuous (i.e. chemostats) and spatially structured (i.e. surface culture) environments, although occasional extinctions have also been reported (Lenski & Levin 1985; Levin & Lenski 1985). Communities of phage and bacteria are often more stable than predicted by theory, and a number of hypotheses have been suggested to explain this observation (Levin & Lenski 1985; Lenski 1988c; Schrag & Mittler 1996). These hypotheses include an ongoing coevolutionary "arms race" between phage and bacteria, and the existence for bacteria of numerical, physiological or spatial refuges from phage attack. Work with the phages T5 and lambda (Schrag & Mittler 1996) supports the idea that spatial refuges (e.g. growth on the walls of culture vessels) may be important in stabilising communities, but this hypothesis cannot explain all observations of stable communities, for example those observed in batch culture (where spatial refuges are reduced because the vessel is replaced daily) (Levin & Lenski 1985). Stability may also be influenced by the productivity of the environment. Bohannan & Lenski (1997) observed that increasing the input of nutrients destabilised the interaction between bacteria and phage, as predicted by ecological theory (Rosenzweig

1971). However, even with increased oscillations, the populations did not go extinct in that study.

The existence of stable communities, the rapid evolution of diversity, and the available knowledge of their genetics and physiology make communities of T-series phages and *E. coli* ideal model systems with which to study the ecology and evolution of communities. In the next section, we will review research on such communities, with an emphasis on the ecology and evolution of bacterial resistance to phage. Subtle variations on the theme of phage-resistance demonstrate and illuminate the various feedbacks between individual interactions, population dynamics, community structure, and evolutionary change. We will focus on simple communities assembled with bacteriophage T4 and *E. coli* B, and those communities assembled with other T-series phages for which there are important differences that have interesting consequences.

THE COMMUNITY ECOLOGY OF T-SERIES PHAGES AND *E. COLI* B

T4 and *E. coli* B: feedbacks between genetic change and community evolution

Mutations that confer resistance to T4 can occur at several loci in E. coli

Resistance to T4 usually occurs through mutations that cause defects in the LPS core of the bacterial outer membrane (Prehm *et al.* 1976; Wright *et al.* 1980). T4 initially binds to the terminal glucose residue of LPS during infection, and changes in the structure or outright loss of this receptor render cells resistant to infection. The loci *lpcA* and *lpcB* are involved in synthesis of the LPS core, as is the set of loci known as *rfa* (Berlyn 1998). Mutations in any of these loci can produce resistance to T4 infection. A number of pleiotropic effects are associated with these mutations, including decreased

competitiveness (Lenski & Levin 1985; Lenski 1988a). Such tradeoffs between predator resistance and competitiveness have also been observed in other organisms (Simms 1992; Grover 1995; Kraaijeveld & Godfray 1997; Fellowes *et al.* 1999; Strauss *et al.* 1999) and have important consequences for community structure.

The cost of resistance varies among mutations, environments and genetic backgrounds

Different mutations that confer equally complete T4-resistance vary in the magnitude of their tradeoff with competitiveness, and in other phenotypic properties such as cross-resistance to other phages (Lenski 1988a) (Fig. 2A). Those mutations that confer cross-resistance to phage T7 tend to be much costlier than those that remain sensitive to this unrelated phage. This pattern occurs because alterations deeper in the LPS molecule have greater effects on the physiology of the *E. coli* cell (Sen & Nikaido 1990), and the initial binding site for T7 is deeper in the LPS core than is the T4 site (Lenski 1988a).

The cost of resistance is also affected by interactions between resistance alleles and the environment (Fig. 2B). For example, the distribution of costs associated with T4-resistance is different when glucose is the growth-limiting resource than when the carbohydrate trehalose is limiting (Bohannon *et al.* 1999). There is also evidence that the cost of T4-resistance depends on temperature (M. Travisano, personal communication).

The cost of T4 resistance also varies with genetic background. For example, mutations that confer resistance to other phages may interact with T4-resistance mutations to influence the net cost. Bohannon *et al.* (1999) observed that it was less costly to be resistant to both T4 and lambda than would be expected from the costs associated with each mutation alone. However, the extent of cost savings depended on the environment in which fitness was measured as well as subtle differences among the mutations. Modifier alleles also interact with resistance alleles to mitigate costs. Lenski (1988b) observed that the cost of T4-resistance declined by almost 50% over 400 generations of evolution in absence of phage, owing to further genetic substitutions that compensated for the maladaptive side-effects associated with T4 resistance.

In summary, the phenotypic costs associated with different resistance alleles depend on gene-gene and gene-environment interactions. These differences in phenotype can profoundly influence higher order properties, such as population dynamics, as we will describe below.

The cost of resistance influences the fate of phage-resistant E. coli mutants

When a T4-resistance mutation first appears, it has two possible fates: it can go extinct or it can persist. The

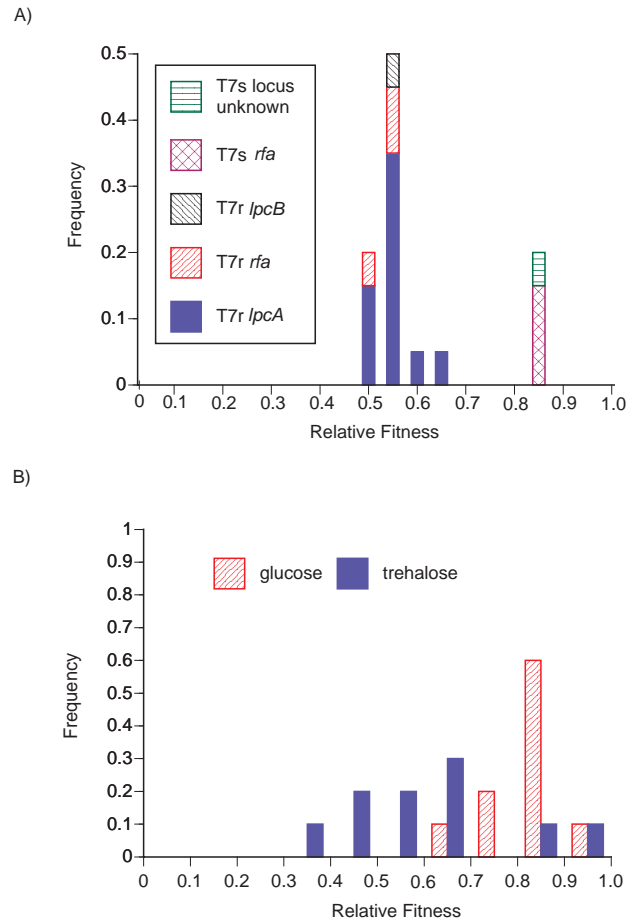


Figure 2 Effects of the mutated gene and the environment on the fitness of phage-resistant *E. coli*. Fitness is measured relative to the phage-sensitive progenitor. (A) Frequency distribution for 20 T4-resistant mutants that vary in genetic locus and cross-resistance phenotype (modified from Lenski 1988a). In the boxed legend, T7r and T7s indicate cross-resistance and sensitivity to phage T7, respectively; names of genetic loci are shown in italics. (B) Frequency distributions for 10 T4-resistant (T7-sensitive) mutants, measured in two different environments.

probability that a cell harbouring a resistance mutation will go extinct is a function of the mutant's cost of resistance (Lenski & Levin 1985) (Fig. 3). In an environment with a constant rate of cell death (such as a chemostat, where washout of cells is equivalent to death), a resistant mutant can persist only if it reproduces fast enough to offset the loss of cells. If the cost of resistance is too high, then the probability of extinction equals one. Even if the mutant type can grow faster than needed to offset losses, there is a substantial chance that it will be lost by random drift (for example, if the first mutant washes out of a chemostat before it has reproduced). The probability of extinction is a function of the cost of resistance (Fig. 3). The higher the cost, the higher the

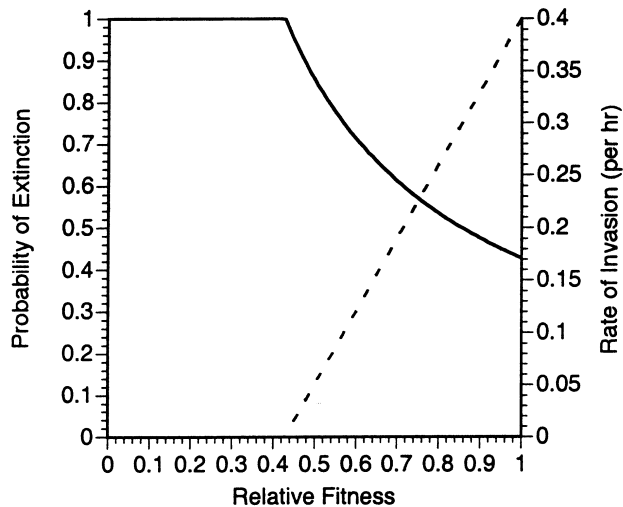


Figure 3 Predicted relationship between relative fitness, probability of extinction (left axis, solid line), and rate of invasion (right axis, dashed line) of phage-resistant mutants (obtained using equations and parameters in Lenski & Levin 1985). The cost of resistance is equal to $1 - W$, where W is the relative fitness of the phage-resistant mutant.

probability of extinction. If a resistant mutant avoids extinction and successfully invades the resident community, then its rate of invasion is also a function of the cost of resistance (Fig. 3). Thus, the fate of each resistance mutation depends on its cost of resistance, which in turn depends on the exact genetic mutation (Fig. 2A) and its interactions with other genes and the abiotic environment (Fig. 2B).

The probability of a resistant mutant's extinction and its rate of invasion (if successful) are also influenced directly by the environment. In a chemostat, both quantities are predicted to be functions of the equilibrium concentration of the growth-limiting resource (Lenski & Levin 1985). This equilibrium concentration, in turn, depends on the input concentration of resource (the environment's "productivity") as well as the demographic parameters of the resident phage-sensitive *E. coli* and the bacteriophage. These predictions are well supported by observations that T4-resistant mutants arise sooner and invade more rapidly in more productive environments than in less productive ones (Bohannan & Lenski 1997).

Invasion by phage-resistant bacteria changes the environment, population dynamics and community structure. Invasion and establishment of T4-resistant mutants has a significant effect on the environment (Bohannan & Lenski 1997). As the resistant bacteria increase in number, the concentration of the limiting resource decreases, stabilising only as the resistant cells reach their resource-limited equilibrium density. The decline in resource

availability caused by the resistant cell population may be significant, especially when the influx of resources into the community is high (Lenski & Levin 1985). Prior to the emergence of the resistant population, the phage hold the sensitive bacteria in check at a density well below their resource-limited equilibrium, so that resources are very abundant; but with the invasion of the resistant population, the equilibrium concentration of limiting resource is drawn down to the "break-even" level that supports just enough growth of the resistant cells to offset washout. The resulting equilibrium concentration of resource is a function of the resource utilisation and growth parameters of the resistant cells, which depend on the cost of resistance. The greater the cost, the higher will be the equilibrium concentration of resource.

It is important to emphasise that the establishment of the resistant population does not cause the extinction of the sensitive population or the phage, provided (i) the resistant mutant suffers at least some cost of resistance in its competitive ability for limiting resource, and (ii) resistance to the phage is absolute. Indeed, all of the T4-resistant mutants we have seen fulfill both conditions (Lenski & Levin 1985; Lenski 1988c; Bohannan & Lenski 1997, 1999). Given these two conditions, sensitive cells have a higher growth rate than the resistant cells when the latter are at their resource-limited equilibrium, which sustains the sensitive cells; the sensitive cells allow the phage to persist; and the phage, in turn, prevent the sensitive cell population from competitively excluding the resistant cells (Levin *et al.* 1977). Indeed, experiments confirm that sensitive bacteria and phage persist after the establishment of the T4-resistant mutant population (Fig. 4).

Despite persistence of both the sensitive bacteria and phage, the decline in concentration of limiting resource that is triggered by the resistant bacteria has significant effects on the other members of the community (Bohannan & Lenski 1997) (Fig. 4). The average density of phage drops significantly and the numerical oscillations of both phage and sensitive bacteria increase in period after the resistant population reaches its equilibrium. The resulting community has also increased in diversity from two "ecospecies" (sensitive *E. coli* and phage) to three (resistant *E. coli*, sensitive *E. coli* and phage). In this new community, the abundance of phage is determined by the growth rate of the sensitive bacteria, which is a function of the equilibrium concentration of limiting resource, which in turn is governed by growth parameters of the resistant mutant; and these parameters depend on the cost of resistance and hence the particular underlying mutation. Thus, the structure of the community (i.e. the abundance of the constituent populations) depends ultimately on the nature of the resistance mutation and its associated cost.

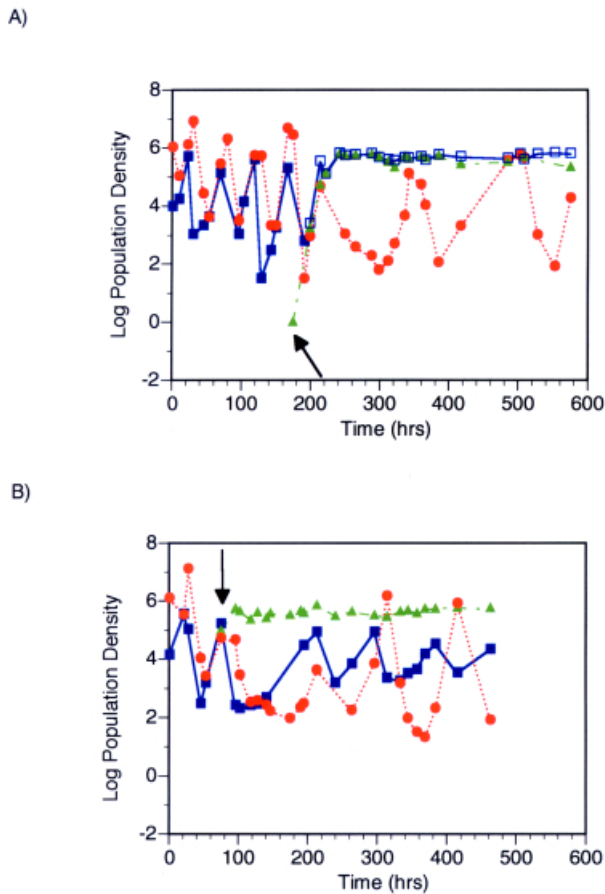
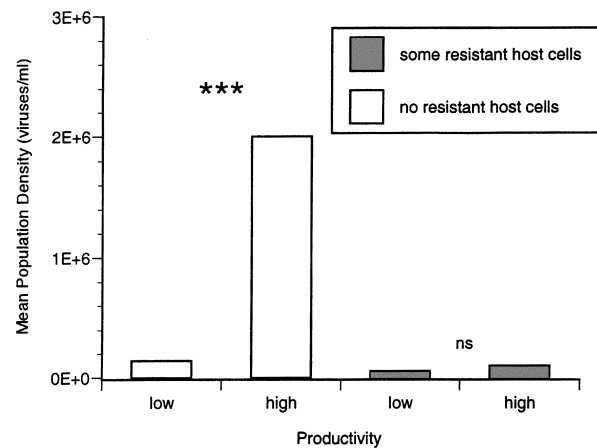


Figure 4 Response of a chemostat community of phage T4 and *E. coli* to invasion by phage-resistant mutants. The population densities (viruses per ml or bacteria per ml) of phage-sensitive *E. coli* (blue squares), phage-resistant *E. coli* (green triangles), and bacteriophage T4 (red circles) have been \log_{10} -transformed. (A) Invasion by a spontaneous mutant. The arrow indicates when the mutant was first detected. Once the resistant mutant invaded the chemostats, it became impossible to track sensitive *E. coli* directly; only the sum of the sensitive and resistant *E. coli* (open squares) was quantified. [From Bohannan & Lenski (1997); Fig. 3(B), with permission of *Ecology*, © 1997 by the Ecological Society of America]. (B) Invasion by an introduced mutant. The arrow indicates when the mutant was experimentally added. The mutant had a selectively neutral genetic marker that allowed us to track the sensitive and resistant populations directly. [From Bohannan & Lenski (1999); Fig. 2(B), with permission of *The American Naturalist*, © 1999 by the University of Chicago].

Invasion by resistant bacteria also affects the community's responses to environmental change

The evolutionary emergence of a resistant population changes not only the community's structure, but also its responsiveness to environmental change (Bohannan & Lenski 1997, 1999; Bohannan 2000). For example, if

A) Phage T4



B) *E. coli*

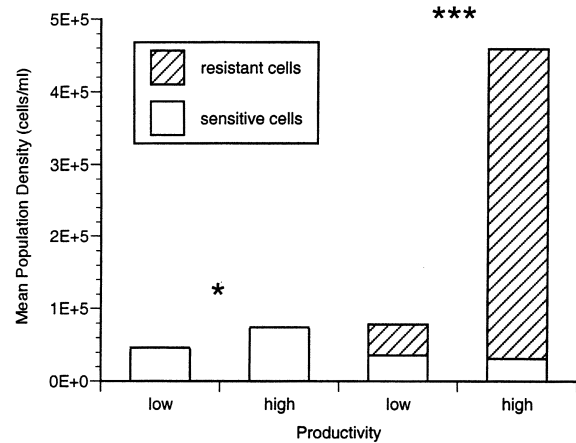


Figure 5 Effect of phage-resistant cells on the response of a chemostat community of phage T4 and *E. coli* to environmental change. Environmental productivity was manipulated by varying the input concentration of glucose, the limiting resource for *E. coli* in this system. Replicate communities consisting of sensitive bacteria and phage, or sensitive bacteria, resistant bacteria and phage, were maintained at each productivity level and population densities were tracked over time. Mean population densities were calculated as the grand mean of the mean population density in three replicate chemostats. (A) Response of the phage population (with and without resistant host cells) to increased productivity (B) Response of the *E. coli* population (with and without resistant individuals) to increased productivity. ns, $P > 0.05$; *, $P < 0.05$; ***, $P < 0.001$ [modified from Bohannan (2000)].

environmental productivity is increased, then a community with only sensitive bacteria and phage responds with a large increase in the phage population (Fig. 5A), a small

increase in the bacteria (Fig. 5B), and a reduction in the dynamic stability of both populations (Bohannan & Lenski 1997). By contrast, a community that also contains resistant bacteria responds to the same increase in productivity with an increase in the resistant population (Fig. 5B), no change in the average density of the phage (Fig. 5A) or sensitive bacteria (Fig. 5B), and reduced stability of the sensitive bacteria and phage populations but increased stability of the resistant population (Bohannan & Lenski 1999). Thus, the response of a given population is determined by the structure of the community in which it is embedded, and that structure in turn is profoundly altered by evolution that depends on a single mutation.

Invasion by phage-resistant bacteria also influences the future evolutionary trajectory of a community. Not only can the evolution of a single resistance mutation alter the interactions between individuals, change the dynamics of multiple populations, affect the structure of a community, and modify the responses of the community to environmental change, but these ecological changes may also feed back to influence the subsequent evolution of the community members. For example, the specific properties of a mutation that confers phage-resistance will determine, at equilibrium, the densities of the phage and resistant bacterial populations and the rate of turnover of the sensitive population (Bohannan & Lenski 1999). These densities and rates will govern, in turn, the production of new mutations in each population and thus its rate of adaptation (Gerrish & Lenski 1998; De Visser *et al.* 1999).

Host-range mutants are not observed in phage T4

In some phage–bacteria interactions, there exist phage mutants with extended host-range, which can infect bacterial mutants resistant to wild-type phage (Lenski 1988c). However, such host-range mutants have not been seen in any of the experimental studies of T4 communities (Lenski & Levin 1985; Bohannan & Lenski 1997, 1999). Evidently, it is very difficult or impossible for phage T4 to counter the evolutionary loss or modification of the *E. coli* B receptor to which it absorbs. As a consequence, the continued persistence of phage T4 in these experimental communities depends on the bacteria's tradeoff between resistance and competitiveness. Lenski (1988b) observed that compensatory mutations could reduce, but did not eliminate, that cost of resistance over the course of 400 generations.

T4 summary

The fact that T4-resistant bacteria are inferior competitors relative to their sensitive progenitors has profound importance for the resulting community structure. More-

over, even subtle genetic differences among resistance mutations affect the magnitude of the tradeoff between resistance and competitive ability. The magnitude of this tradeoff determines whether a resistant mutant can invade the community and what effects its invasion has on the population dynamics and community properties, in particular the equilibrium level of the limiting resource. The population dynamics set the stage on which the evolutionary play will continue in the future, thus constraining or promoting adaptation of the various interacting members. The environment, in particular the concentration of growth-limiting resource, is the key component in this process; it both acts as a catalyst for, and is affected by, evolutionary changes in the community.

T7 and *E. coli* B: coevolution and the asymmetry of arms races

*Coevolution between phage T7 and *E. coli* depends on the nature of resistance mutations*

Phage T7 is phylogenetically unrelated to phage T4 but they both adsorb to moieties in the LPS core on the surface of *E. coli* B (Goldberg *et al.* 1994), and mutations that confer resistance to T4 often give cross-resistance to T7 (Fig. 2A). Moreover, experimental communities of T7 and *E. coli* B behave similarly in many ways to communities of T4 and *E. coli* B (Chao *et al.* 1977; Lenski & Levin 1985). However, one important difference is that host-range mutants often occur in T7 but not in T4 (Chao *et al.* 1977). This difference gives rise, at the level of the community, to a sort of ‘‘arms race’’ between resistance mutations in *E. coli* and host-range mutations in T7 (Lenski & Levin 1985). That is, bacterial mutants resistant to wild-type phage invade, followed by host-range mutants of the phage that can infect both the sensitive and resistant *E. coli*. A second-order *E. coli* mutant appears that is resistant to both the wild-type and host-range mutant phage, potentially re-starting the coevolutionary cycle. Chao *et al.* (1977) observed that three bacteria populations (sensitive, resistant, and second-order resistant) could coexist with two phage populations (wild-type and host-range mutant) with one exogenous resource (glucose). This complexity implies a tradeoff in both populations: between resistance and competitive ability for glucose in the bacteria, and between extended host-range and competitive ability for more sensitive hosts in the phage.

The coevolutionary arms race is asymmetrical

Despite the potential for T7 mutants with extended host-range, the coevolutionary arms race seems to favour the bacteria. That is, *E. coli* B eventually produces a resistance

mutation that phage T7 cannot overcome with a corresponding host-range mutation (Lenski & Levin 1985). This asymmetry apparently occurs because bacterial mutants can resist T7 infection by either modifying or eliminating the receptor molecule, whereas T7 can overcome the former but not the latter mechanism. Which type of mutation appears first in the bacterial population may determine whether a coevolutionary response occurs in the phage population.

Despite this asymmetry, T7 persists even after a resistant mutant has evolved that it cannot attack, and it does so because the phage-sensitive bacterial progenitor is competitively superior to the resistant mutants (Lenski & Levin 1985). Over a longer evolutionary time span, it is not clear what might happen to these communities, but two interesting possibilities can be imagined. First, the cost of resistance may decline as modifier mutations arise (as described for T4 above). If the cost of resistance is eventually eliminated, then the resistant bacteria would exclude their sensitive counterparts and the phage would also go extinct. The other possibility is that a new host-range mutant phage might eventually arise that is capable of using a different receptor on the surface of the *E. coli* B cell, and the coevolutionary cycle could begin anew. Such switching of receptors has been observed in certain other phages (Hashemolhosseini *et al.* 1994), but whether it can occur in T7 is unknown.

T7 summary

Coevolution between *E. coli* B mutants that are phage-resistant, and phage T7 mutants with extended host-range, can lead to higher diversity of both phage and bacterial types, although eventually resistant bacteria arise that no mutant phage can infect. The duration of this coevolutionary phase depends on the specific resistance mutations in the bacteria, some of which can be countered by phage host-range mutations and some of which cannot.

T2 and *E. coli* B: predator-mediated indirect interactions

Partial and complete resistance to T2 in E. coli

Bacteriophage T4 adsorbs to just one kind of receptor on the surface of *E. coli* B. When production of that receptor is blocked by a mutation, the resulting bacterium is completely resistant to T4 infection (Lenski & Levin 1985). Phage T2 differs in that two receptors are involved in its initial binding to *E. coli* B cells (Lenski 1984). One of these receptors is the same glucose residue in the LPS used for adsorption by T4. The mutational loss of this receptor reduces the rate at which T2 infects *E. coli* by approximately 50% (Lenski 1984). Thus, mutants of *E. coli* that have partial resistance to T2 can be found by

screening for the correlated trait of absolute resistance to T4. Once *E. coli* B has evolved partial resistance to T2, it then becomes much easier to isolate secondary bacterial mutations that confer complete resistance to T2 (Lenski 1984). Partial resistance may also occur against other phages, but it is not commonly observed, presumably because it is a more subtle phenotype (difficult to detect by classical methods) and because it is less advantageous to bacteria evolving with phage (unlikely to emerge in chemostat experiments). However, the ability to detect partially resistant mutants to T2 by their cross-resistance to T4, coupled with the fact that it is difficult to generate complete resistance to T2 in a single mutational step, means that the ecological and evolutionary consequences of both partial and complete resistance can be studied with phage T2 (Bohannan & Lenski 2000).

Partial resistance leads to apparent competition

“Apparent competition” refers to the indirect interaction between populations that share a common predator (Holt 1977). Predator abundance is determined by the total prey availability, and one prey population can intensify predation on another prey population by causing an increase in predator abundance. When a mutant prey evolves that is completely resistant to predation, the only interaction between the resistant and sensitive prey populations is their competition for shared resources. But when a mutant prey emerges that is only partially resistant to its predator, the partially resistant and sensitive populations interact both through their shared predator and through their shared resources.

For example, when a bacterial mutant emerges that is fully resistant to T4, it escapes from population control by the phage predator and its density becomes resource-limited. By contrast, when a mutant emerges that is only partially resistant to T2, the mutant population remains under regulation by the phage. And because the partially resistant prey are less vulnerable, the density of predators necessary to hold them in check can be greater than the density that sufficed to control the sensitive prey population. Thus, the appearance of a partially resistant population can cause an increase in T2 abundance and thereby intensify predation pressure on the sensitive population (Levin *et al.* 1977; Lenski 1984).

Whether this indirect interaction between partially resistant and sensitive *E. coli* results in exclusion of the sensitive population depends on the productivity of the environment (Bohannan & Lenski 2000). At higher resource levels, the population density of phage T2 supported by the partially resistant population exceeds that level which allows the sensitive population to persist, thus causing exclusion of the sensitive population as predicted by ecological theory (Levin *et al.* 1977; Holt *et*

al. 1994; Leibold 1996). By contrast, at low resource levels, the sensitive bacteria appear to gain the upper hand, owing to their advantage in competition for the limiting resource, again consistent with theory. Thus, when the molecular basis of the interaction between bacteria and phage is such that partial resistance is an important feature, the community structure and its dependence on environmental productivity are quite distinct from the situation that prevails with complete resistance.

The cost of resistance determines the effect of invading resistant mutants on community structure

Communities of phage T2 and *E. coli* B eventually evolve both partial and complete resistance (Lenski 1984; Bohannon & Lenski 2000). Partial resistance usually emerges first, followed by complete resistance (Fig. 6). The resulting structure of the community depends not only on the critical distinction between partial and complete resistance (as described above), but also on the competitive costs associated with specific mutations that are responsible for the resistant phenotypes (Bohannon & Lenski 2000).

Once a completely resistant mutant has emerged, it will become resource limited and can coexist with another bacterial population that is limited by the phage. Will the

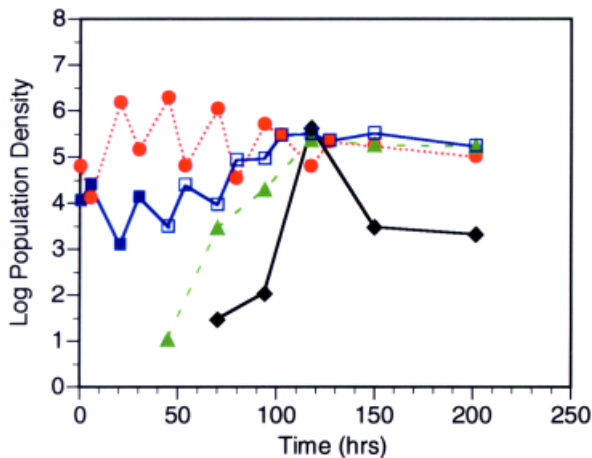


Figure 6 Invasion of a chemostat community of phage T2 and *E. coli* by partially resistant and completely resistant bacterial mutants. The population densities (viruses per ml or bacteria per ml) of bacteriophage T2 (red circles), sensitive *E. coli* (blue squares), partially resistant *E. coli* mutants (green triangles), and completely resistant *E. coli* mutants (black diamonds) are log-transformed. After the mutants invaded, only the total *E. coli* population (open squares) could be quantified. [From Bohannon and Lenski (2000, Fig. 8C), with permission of *The American Naturalist*, © 2000 by the University of Chicago].

coexisting phage-limited population be the partially resistant mutant or its fully sensitive progenitor? The answer hinges on the cost of complete resistance, which determines the equilibrium concentration of resources available to other bacteria and hence the outcome of resource competition and apparent competition between the sensitive and partially resistant populations. Imagine, for example, two different completely resistant mutants, one with a lower cost than the other (Fig. 7). The hypothetical mutant with the low cost of resistance holds the resource to a lower equilibrium level, creating an environment in which the sensitive *E. coli* can outcompete the partially resistant population because the sensitive *E. coli* are better competitors for resource; in this case, the sensitive population should persist and the partially resistant type become extinct (Fig. 7A). In contrast, the equilibrium resource concentration should be higher when the completely resistant mutant bears a high cost of resistance, a situation wherein the partially resistant population can outcompete the sensitive population because the partially resistant population is less vulnerable to attack by phage; in this case, the partially resistant *E. coli* should exclude the fully sensitive type (Fig. 7B). [Of course, if the cost of resistance is too high, then the completely resistant mutant cannot invade. In that case, the fate of the partially resistant and sensitive populations depends on the gross productivity of the environment, as opposed to the net productivity after depletion by the completely resistant population.] Indeed, different community structures have been observed after invasion of the completely resistant type, although it remains to be investigated whether these community differences are caused by resistance mutations with different costs (Bohannon & Lenski 2000).

Host-range mutants of T2 are also observed

As described earlier for phage T7, mutant T2 sometimes emerge that have extended host-range and so can infect bacterial mutants that are completely resistant to wild-type T2 (Lenski & Levin 1985). However, bacterial mutants that are resistant even to these extended host-range mutants eventually emerge, as they did in the case of T7, and no further host-range extensions are observed (Lenski & Levin 1985). Thus, in all of the experimental phage communities examined, there seems to be a similar asymmetry in the “arms race” between resistant mutants in the *E. coli* prey and host-range mutants in the bacteriophage predators, such that some resistance mutations cannot be countered by host-range mutations; as a consequence, the phage depend for their long-term persistence on the tradeoff between resistance and competitiveness in their prey (Lenski & Levin 1985).

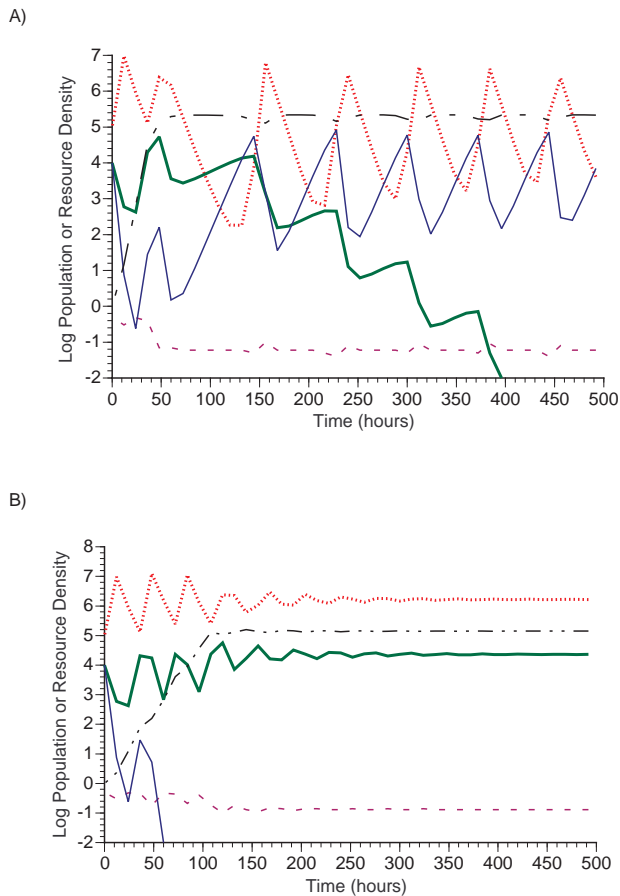


Figure 7 Predicted response of a glucose-limited chemostat community of phage T2, sensitive *E. coli*, and partially resistant *E. coli* to invasion by completely resistant mutants with different costs of resistance. The predicted population densities of phage (red dotted line), sensitive *E. coli* (blue solid line), partially resistant *E. coli* (green bold line), completely resistant *E. coli* (black dotted-dashed line), and the concentration of glucose (purple dashed line) are all log-transformed. See Appendix for details of the model and simulation, and Table 2 for parameters. (A) Completely resistant mutant with a low cost (high fitness). Fitness relative to the phage-sensitive progenitor is 0.5067. (B) Completely resistant mutant with a high cost (low fitness). Fitness relative to the phage-sensitive progenitor is 0.2816.

T2 summary

Different bacterial mutations confer either partial or complete resistance to phage T2 attack. In the case of partial resistance, unlike complete resistance, the mutant bacteria interact with sensitive bacteria through their shared predator, as well as through shared resources. This predator-mediated interaction gives rise to the phenomenon of apparent competition, such that

the partially resistant mutant excludes the sensitive type in high productivity environments. Thus, there is a complex interplay between genetic factors (which determine whether resistance is partial or complete) and environmental factors (which govern productivity) in determining the trajectory of community evolution. Subsequent evolution of the partially resistant bacteria yields mutations that are completely resistant to phage T2, and these mutations then lead to alternative community structures depending on their competitive costs.

T5 and *E. coli* B: the importance of tradeoffs

E. coli can become resistant to T5 without incurring any competitive cost

As explained above, phages T4, T7 and T2 all depend for their long-term persistence on the tradeoff between resistance and competitiveness in their bacterial prey. By contrast, mutants of *E. coli* B that are completely resistant to T5 do not have a competitive cost under the environmental conditions used in laboratory experiments to date; moreover, T5 (like T4) does not produce any mutants with extended host range (Lenski & Levin 1985). As a consequence, T5-resistant mutants drive their sensitive progenitors, and with them phage T5, to extinction (Lenski & Levin 1985). The absence of any cost of resistance precludes predator-mediated coexistence of sensitive and resistant populations.

The example of T5 is interesting in three respects. First, it provides a sort of “negative control” for the hypothesis that the persistence of the other phages (following the evolution of completely resistant mutants that cannot be overcome by host-range mutants) depends on the tradeoff between resistance and competitive ability. Such persistence was observed for all three phages, as was the theoretically requisite tradeoff. Phage T5 provides the exception that tests, and confirms, the rule: no such tradeoff exists, and the phage goes extinct. Second, this finding begs the question of how phage T5 persists in nature. There are several possibilities that depend, one way or another, on a tradeoff between resistance and competitive ability. Perhaps some T5 hosts, owing to different genetic backgrounds, do suffer a tradeoff, or perhaps the receptor for T5 is important for competitive fitness in certain environments but not others. Third, as we will discuss in the section on Future Directions, the case of T5 might represent a local “endpoint” for all phages if, through the evolution of modifier genes, the resistant bacteria could eliminate the cost of resistance in their local environment.

T5 summary

Mutations in *E. coli* B that confer complete resistance to phage T5 do not impose any associated cost in terms of competitive fitness, nor does T5 counter these resistant bacteria by host-range mutations. Therefore, unlike any of the other phages studied, phage T5 quickly goes extinct in these experimental communities.

SYNTHESIS

A major goal of community ecology is to link biological processes at lower scales with community patterns. Microbial communities are especially powerful model systems for making these links. The life cycles and interactions of bacteria and lytic phage (Fig. 1) are basically the same for all four cases reviewed here, as are two ecological features: (1) the phage are able to hold the density of sensitive bacteria in check at a level below that which the resources allow in the absence of phage; and (2) mutant bacteria evolve that are more resistant to phage attack than their progenitor. But several important differences among these interactions emerge that have dramatic effects on the resulting community ecology: (3) whether bacterial resistance to the phage is partial or complete; (4) whether phage mutants with extended host-range evolve that can counter resistance mutations; (5) whether bacterial resistance to phage engenders a cost, or tradeoff, in terms of reduced competitiveness for limiting resources; and (6) the magnitude of the cost of resistance. All these issues reflect subtle differences in the molecular and genetic bases of the phage–bacteria interactions, yet their effects ramify through the population dynamics and lead to widely divergent community structures and evolutionary trajectories.

That such low-level details can profoundly influence ecological communities may seem disheartening at first, especially for those researchers who study more complex organisms. But we prefer a more optimistic view, one with two parts. First, as biologists, it is intriguing to see how such apparently minor differences in species interactions can profoundly impact ecological processes and patterns. Second, all of the divergent evolutionary outcomes can be understood within the broad theoretical framework that ecologists have developed to understand population processes and community structure. That is, the mathematical theories of community ecology have already addressed and drawn attention to the general issues highlighted under points (3) to (6) above. The theories can accommodate such divergent cases because, although the differences ultimately depend on genetic and molecular details, the effects of these differences are exerted through parameters that govern ecological interactions between populations and their resources. Thus,

the environment, and especially the concentration of growth-limiting resources, acts as a conduit through which genetic changes in any one population affect the ecology and evolution of other populations.

FUTURE DIRECTIONS

Bacteria and phage have proved useful subjects for studying the ecology and evolution of communities, especially in testing theoretical models of community “modules” and illuminating ways in which subtle differences in interactions can profoundly influence population processes and community structures. Three new areas that we believe are particularly promising for future research with bacteria and phage include: (1) consequences of long-term community evolution; (2) effects of spatial structure on the ecology and evolution of communities; and (3) effects of trophic complexity on the ecology and evolution of communities.

Long-term community evolution

The studies we have reviewed clearly show that a key factor in the evolution of these communities is whether bacterial resistance to phage imposes a cost in terms of reduced competitiveness for resources. Such costs were observed for resistance to phages T4, T7, and T2, but not for resistance to phage T5, and as a consequence only T5 was observed to go extinct following the evolution of resistance. But while such costs appear to be commonplace, other studies have shown that bacteria can often evolve modifiers that reduce the deleterious effects, or side-effects, of many mutations, including those that confer resistance to phage (Lenski 1988b; Moore *et al.* 2000). If, through the evolution of modifier genes, bacteria populations could eventually eliminate the cost of being resistant to phage, then the tradeoff-free resistant bacteria would drive the sensitive bacteria and the phage extinct, at least locally (i.e. in an environment in which the modifiers eliminated the entire cost).

Under this scenario, the community’s evolutionary trajectory would initially increase its diversity, as resistant mutants appear and invade and the phage respond coevolutionarily with any host-range mutants. However, this first flush of adaptive radiation would be followed by a period of decelerating coevolution, as resistance mutations appear with lower cost (reducing the size of the phage population and thus its rate of evolution), and as resistant mutations appear that cannot be countered by host-range mutations. This middle phase would be followed by a sudden “mass extinction” when a resistant mutant appeared that no longer had a cost in terms of reduced competitive ability. All of the

components in this scenario have been observed experimentally, except that modifiers have so far been found that can reduce—but do not eliminate—the cost of phage resistance (Lenski 1988b). Phage T4 has been observed to coexist with *E. coli* for a full year (Horne 1970), which implies that complete compensation for the cost of resistance, if it is possible at all, requires even more time. Thus, long-term studies of the evolution of phage and bacteria communities are needed to determine the likelihood of this scenario. While difficult, such studies could provide fascinating insights into the evolution of biological communities.

Spatially structured environments

Microbial model systems have been used to explore the role of spatial structure in several studies, including the ecology and evolution of allelopathy (Chao & Levin 1981), the stability of predator–prey interactions (Schrag & Mittler 1996), and adaptive radiation (Korona *et al.* 1994; Rainey & Travisano 1998). Interest in the role of spatial structure in the ecology and evolution of communities has recently grown dramatically, owing at least in part to the development of spatially explicit ecological models (Keeling 1999). Many of these models could be put to the test experimentally using simple communities of bacteria and phage. Moreover, several important issues could be addressed with this approach, including the relationship between spatial structure and the evolution of virulence (Boots & Sasaki 1999) and the effects of spatial structure on the maintenance of biodiversity (Hochberg & Hawkins 1993).

Trophic complexity

One important advantage of phage–bacteria systems is that the initial complexity of a community is under the control of the experimenter. Trophic complexity can be reduced to a minimum (one predator type and one prey type) and then increased incrementally to examine its effects on population dynamics, community properties, and evolutionary change. Both the number of trophic interactions and the nature of those interactions can be manipulated in such systems. For example, until fairly recently, most models of consumers and prey had a single consumer population (in contrast to models with abiotic resources, which have examined multiple consumers for much longer: see Tilman 1982). However, interest has been growing in community modules that contain multiple consumer populations (Begon & Bowers 1995), which have led to pathogen–pathogen–host models (Hochberg & Holt 1990), pathogen–parasitoid–host models (Hochberg *et al.* 1990), and

parasite–predator–host models (Anderson & May 1986).

These models' predictions could be explored using communities with multiple phages as well as with combinations of phage and protist consumers. Issues that may be addressed include conditions for the coexistence of multiple parasitoid (phage) types on one host, and the effects of interactions between pathogens (chronic phage), parasitoids (lytic phage), and predators (protists) on population dynamics, community structure and evolutionary change. Bacteria and phage may also be used to study the effects on communities of combining various other types of interaction. Ecological interactions other than competition and predation have received less attention from experimental ecologists. For example, commensalism (e.g. bacteria feeding on the exudate of other bacteria), mutualism (e.g. microbes exchanging resources), and allelopathy (e.g. bacteria inhibiting the growth of other bacteria) could be added to simpler communities of bacteria and phage in order to study the separate and combined effects of multiple interactions on populations, communities, and their evolutionary trajectories.

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APPENDIX

To predict the response of the community containing phage T2 and sensitive *E. coli* B to invasion by partially and completely resistant mutants, we modelled the community by modifying the model developed by Levin *et al.* (1977). We examined the numerical behaviour of our model using STELLA II simulation software (High Performance Systems 1994).

Mathematical model

The model consists of five coupled differential equations:

$$\begin{aligned} dR/dt &= (R_0 - R)\omega - \varepsilon N_A \psi_A R / (K_A + R) \\ &\quad - \varepsilon N_B \psi_B R / (K_B + R) - \varepsilon N_C \psi_C R / (K_C + R) \\ dN_A/dt &= N_A \psi_A R / (K_A + R) - \alpha_A N_A P - \omega N_A \\ dN_B/dt &= N_B \psi_B R / (K_B + R) - \alpha_B N_B P - \omega N_B \\ dN_C/dt &= N_C \psi_C R / (K_C + R) - \omega N_C \end{aligned}$$

Table 1 Symbols used in the mathematical model given in the Appendix

Symbol	Definition
R	concentration of glucose in the chemostat
N_A	population density of sensitive bacteria
N_B	population density of partially resistant bacteria
N_C	population density of completely resistant bacteria
P	population density of phage
R_0	concentration of glucose in the reservoir
ω	flow rate through the chemostat (turnover rate)
ε	growth efficiency (reciprocal of the bacterial yield)
ψ_A	maximum growth rate of sensitive bacteria
ψ_B	maximum growth rate of partially resistant bacteria
ψ_C	maximum growth rate of completely resistant bacteria
K_A	resource concentration at which sensitive bacteria grow at one half ψ_A
K_B	resource concentration at which partially resistant bacteria grow at one half ψ_B
K_C	resource concentration at which completely resistant bacteria grow at one half ψ_C
α_A	attack (i.e. adsorption) rate of phage on sensitive bacteria
α_B	attack (i.e. adsorption) rate of phage on partially resistant bacteria
β	burst size of phage (progeny produced per infected cell)
τ	latent period of phage (time elapsed between infection and burst)
$e^{-\tau\omega}$	fraction of bacteria infected at time $t - \tau$ that has not washed out before lysing
N_A'	density of sensitive bacteria at time $t - \tau$
N_B'	density of partially resistant bacteria at time $t - \tau$
P'	density of phage at time $t - \tau$

$$\frac{dP}{dt} = \beta e^{-\tau\omega}(\alpha_A N_A' P) + \beta e^{-\tau\omega}(\alpha_B N_B' P) - \alpha_A N_A P - \alpha_B N_B P - \omega P.$$

In these equations, R is the concentration of glucose in the chemostat vessel; N_A , N_B and N_C are densities of sensitive, partially resistant, and completely resistant bacteria, respectively; and P is the density of phage T2. The model parameters are defined in Table 1. Those parameters that are specific to the sensitive bacteria are denoted by subscript A; those that are specific either to partially or completely resistant bacteria are indicated by subscripts B or C, respectively. Note that equations describing the dynamics of infected bacteria (which are fated to die) could also be written; the inclusion of these infected cell populations has little effect on the dynamics of the other populations.

This model assumes an open and completely mixed environment. Growth-limiting resource flows into the environment at concentration R_0 and rate ω . Phage, bacteria and unutilised resource flow out of the habitat at this same rate. Bacteria multiply at a per capita rate that is a hyperbolic function of the resource concentration in the environment ($\psi R / \{K + R\}$) and each cell replication consumes ε of the resource. Phage attack and kill bacteria at a per capita rate that is a linear function of the bacterial density (αN). Each attack is lethal and results in the release of β phage progeny after a time lag of τ . Phage

reproduce at a rate that is a function of the number of progeny produced from each *E. coli* attacked (β), the fraction of bacteria attacked at time $t - \tau$ that has not flowed out of the chemostat before the phage progeny are released ($e^{-\tau\omega}$), the attack rate at time $t - \tau$ ($\alpha N'$), and the phage density at time $t - \tau$ (P'). The completely resistant cells (N_C) are assumed to not be “handled” by the phage or otherwise interfere with the consumption of sensitive and partially resistant cells.

The parameter values used for producing Fig. 7 are given in Table 2. Biologically reasonable demographic parameters were assumed for the completely resistant populations. The relative fitness of a completely resistant mutant must be less than the relative fitness of a partially resistant mutant and yet large enough that the completely resistant mutants can invade. Partially T2-resistant mutants have been reported to have an average relative fitness of 0.5750 (Bohannan and Lenski 2000); 0.2589 is the minimum relative fitness that would allow a positive rate of invasion under the conditions assumed in this model (calculated using equations from Lenski & Levin 1985). The demographic parameters chosen for the completely resistant populations in this model result in relative fitnesses (0.2816 for the population with a high cost, and 0.5067 for the population with a low cost) that meet these requirements.

Table 2 Parameter values used with the model in the Appendix to produce Fig. 7

Symxbol	Parameter value	Source of estimate
R_0	0.5 $\mu\text{g ml}^{-1}$	(Bohannan & Lenski 2000)
ω	0.2 h^{-1}	(Bohannan & Lenski 2000)
ε	2×10^{-6} μg	(Bohannan & Lenski 1997)
ψ_A	0.7726 h^{-1}	(Vasi <i>et al.</i> 1994)
ψ_B	0.7027 h^{-1}	(Bohannan & Lenski 1999)
ψ_C	Either 0.5 (high cost) or 0.7 (low cost) h^{-1}	Assumed for model
K_A	0.0727 $\mu\text{g ml}^{-1}$	(Vasi <i>et al.</i> 1994)
K_B	0.123 $\mu\text{g ml}^{-1}$	(Bohannan & Lenski 1999)
K_C	Either 0.2 (high cost) or 0.15 (low cost) $\mu\text{g ml}^{-1}$	Assumed for model
α_A	2×10^{-7} ml h^{-1}	(Lenski 1984)
α_B	1×10^{-7} ml h^{-1}	(Lenski 1984)
β	98 viruses per cell infected	(Levin <i>et al.</i> 1977)
τ	0.5 h	(Levin <i>et al.</i> 1977)

Numerical simulations

We ran the numerical simulations in Fig. 7 using a time step of 0.05 h. We tested the sensitivity of these simulations to step size by also running replicate simulations at step sizes of 0.1, 0.05 and 0.025 h. Varying the size of the time steps had no detectable effect on the results. For purpose of graphical illustration, we “sampled” the output of simulations every 12 h, which is the approximate sampling interval used in our experiments.

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