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Dynamics of Interactions between Bacteria and Virulent Bacteriophage

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1. Introduction

The interactions of bacteria and their viruses (bacteriophage) are, by and large, ones of trophic exploitation. In fact, "phage" is derived from the Greek word for "devour." Using the criterion of relative size, the interactions can be defined as parasitism (Bull and Slater, 1982). Because replication by most virulent phage necessarily results in bacterial death, these interactions could also be called predation. Certain interactions could even be termed mutualistic, as some temperate phage encode phenotypic characteristics that are of direct benefit to their hosts. Semantics aside, the fundamental ecological question that I will attempt to address in this chapter is: What role do bacteriophage infections play in limiting the abundance of bacteria?

Such a broad question cannot be answered using any single approach or line of evidence. Therefore, I have chosen to organize this chapter in a hierarchical manner, moving from mathematical models, through simple laboratory communities, and finally to much more complex communities in natural settings. But first it is necessary to review the basic biological features of the interactions between bacteria and phage, as revealed by the extraordinary advances in the areas of microbial genetics and molecular biology. This research provides a precise methodological and conceptual framework for examining ecological hypotheses, probably unrivaled for any other parasite–host interaction. The same features of

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the phage–bacteria system that have been so valuable in nonecological research also contribute to its power in addressing fundamental ecological questions: specifically, ease of culture and sampling, high population densities, and short generation times. In fact, generations are so short that it becomes imperative to consider the effects of evolutionary change on population dynamics, even over the course of short-term experiments.

2. Molecular and Genetic Bases of the Interaction

There are a number of excellent references on the basic features of the interaction between bacteria and phage. Stent (1963) provides a historical perspective on the progress in elucidating the bases of the interaction, but this book is now somewhat dated. Luria *et al.* (1978) provide a more up-to-date introduction to phage biology. Mathews *et al.* (1983) and Hendrix *et al.* (1983) provide comprehensive summaries of recent research on virulent coliphage T4 and temperate coliphage Lambda, respectively, emphasizing the regulation and expression of phage genes.

2.1. The Course of Lytic Infections

2.1.1. Virulent and Temperate Phage

Bacteriophages are generally divided into two basic classes, virulent and temperate. For both, infection of a host bacterium commences with the adsorption of the phage to the bacterial surface, followed by the introduction of the the genome of the phage into the bacterium. For virulent phage, there follows a period of vegetative growth during which time its genome is replicated and encapsulated intracellularly, terminating with lysis of the bacterium and the release of infective progeny phage (Ellis and Delbruck, 1939). Temperate phage infection may also proceed via this lytic process, or the infecting phage may form a semistable association with the bacterium, a phenomenon known as lysogeny (Lwoff, 1953). In the event of this lysogenic response, the phage genome (referred to as a prophage) is replicated along with the bacterial genome, and is inherited by each of the daughter cells (referred to as lysogens). Lysogens are immune to reinfection by the same temperate phage, although they are not usually immune to virulent mutants of this phage. After one or more generations of lysogenic replication, the prophage may be stimulated to enter the lytic cycle (induction), or it may be lost from the genome of the bacterium during subsequent replication (segregation). The distinction between virulent and temperate phage is thus made on functional grounds, with virulent phage capable only of the lytic mode of replication and temperate phage capable of both the lytic and lysogenic modes of replication.

The existence of temperate phage raises a number of interesting questions that will not be addressed in this chapter. What selective pressures are responsible for the evolution of lysogeny (Levin and Lenski, 1983, 1985; Stewart and Levin, 1984)? What effect does prophage carriage have on the competitive ability of lysogens (Dykhuizen and Hartl, 1983)? What conditions determine the outcome of the facultative "decision" by a temperate phage to lyse or lysogenize its host (Echols, 1972)? What role do phage, especially temperate, play in the infectious transfer of bacterial genes (Reanney, 1976; Reanney *et al.*, 1983)? Furthermore, I will not consider the actual phylogenies of bacterial viruses, nor the role that recombination may have played in this phylogenetic evolution (Bradley, 1967; Botstein, 1980; Reanney and Ackermann, 1982; Campbell and Botstein, 1983).

While the focus of this chapter is on the interaction of virulent phage and their hosts, the distinction between temperate and virulent is somewhat artificial. The repressor protein responsible for the maintenance of lysogeny is coded for by the genome of the temperate phage, but this response is also dependent on environmental and genetic factors acting on the host bacterium. Thus, the classification of a phage as temperate or virulent may depend on the particular host or environment in which the lysogeny criterion is tested. Nor can the distinction be made on phylogenetic grounds; with one or a few mutations, temperate phage can lose their ability to form lysogens and hence become virulent phage (Lwoff, 1953; Bronson and Levine, 1971; Ptashne *et al.*, 1980). Even for phage clearly identifiable as temperate, the likelihood of the lysogenic response for any given infection is usually much lower than the likelihood of the lytic response.

Moreover, certain types of phage infections are not readily classified as either lytic or lysogenic. For example, the normally virulent coliphage T3 may replicate alongside the bacterial genome for several generations before lysing the infected cells (Fraser, 1957). This response differs from true lysogeny, however, in that this pseudolysogenic state is not maintained by a phage-encoded repressor protein (Kruger and Schroeder, 1981). Similarly, normally virulent phage of *Bacillus subtilis* may persist in living cells through the sporulation process (Barksdale and Arden, 1974). The distinction is even more problematic for the filamentous phages, which are released continuously from growing and dividing cells. The growth rate of bacteria is usually reduced by infection with these phage, but lysis does not normally occur (Marvin and Hohn, 1969). Asso-

ciations between filamentous phage and their hosts are perhaps more analogous to "typical" host–parasite associations, in contrast to the dormant lysogenic state and the lethal lytic response.

2.1.2. *Adsorption*

Phage and bacteria encounter one another through random Brownian motion. The adsorption process commences with the binding of the phage adsorption organelle to highly specific receptor sites on the bacterial surface. Sites of attachment vary from bacterium to bacterium and from phage to phage. A wide variety of moieties at the cell surface may serve as receptors for particular phage, including proteins (Schwartz, 1980), lipopolysaccharide in Gram-negative bacteria (Wright *et al.*, 1980), and peptidoglycan and teichoic acid in Gram-positive bacteria (Archibald, 1980). It is not uncommon for two or more different phage to recognize the same receptor sites on a particular bacterium (Schwartz, 1980), and a few phage may even be capable of adsorbing to two or more different receptors (Morona and Henning, 1984, 1986).

Not surprisingly, the receptor sites on the bacterial surface serve particular functions, and have been exploited secondarily by bacteriophage. For example, many of the proteinaceous receptor sites are involved in the transport of specific nutrients, including sugars, amino acids, and vitamins (Braun and Hantke, 1977). Other receptors are associated with organelles of specific function, such as flagella and conjugative pili, the latter serving as adsorption sites for the so-called male-specific phages.

The attachment of the phage adsorption organelle to bacterial receptors is initially reversible (Goldberg, 1980). That is, the phage may be dissociated, for example, by diluting the reaction mixture or killing the bacteria, with the phage retaining their infectious capacity. The association eventually becomes irreversible, and this irreversibility is linked to the steps leading to the penetration of the genetic material of the phage into the bacterial cell.

The kinetics of the adsorption process can be studied by means of an approach pioneered by Krueger (1931) and Schlesinger (1932). Known densities of bacteria and phage are combined, usually with fewer phage than bacteria (i.e., a low multiplicity of infection, or MOI), so that complications arising from multiple phage adsorptions to a single bacterium can be ignored. If the rate of irreversible adsorption of phage to bacteria is sufficiently high, it is possible to measure directly the decline in free (i.e., unadsorbed) phage. At frequent intervals, a subsample of the culture is diluted and chloroformed (Adams, 1959). Dilution effectively stops the

cell density-dependent process of phage adsorption, while chloroform kills bacteria and phage that have adsorbed irreversibly to the bacteria, but does not usually affect free phage. The duration of the experiment must be limited to preclude the formation of complete phage progeny within those cells that were infected first (see discussion of the eclipse period in Section 2.1.3). If the proportional decline in free phage is slight, then more complex procedures are necessary. For example, using antiserum active against the phage, it is possible to remove free phage and thus measure directly the increase in infected bacteria (Adams, 1959).

The dynamics of phage adsorption are usually described empirically by a first-order rate constant, which is expressed as unit volume per unit time. This rate can be calculated from the slope of the exponential decline in concentration of free phage divided by the density of bacteria on which adsorption took place. Departures from first-order kinetics become apparent at very high densities of bacteria (Stent and Wollman, 1952). In such cases, the rate of phage adsorption reaches some maximum that is independent of bacterial density, as the kinetics are limited not by the formation of reversible attachments, but by the formation of irreversible associations. It has been found that the rate of phage adsorption to bacteria under favorable conditions can be very close to the rate of collision by Brownian motion (Schlesinger, 1932; Delbruck, 1940a; Schwartz, 1976). Thus, the attachment of an adsorption organelle of a phage to receptor sites on a bacterium can be an extremely efficient process.

The kinetics of adsorption can also be affected at higher multiplicities of phage. In extreme cases, one may observe the competition of phage for limiting receptor sites. A large number of adsorptions to an individual bacterium may also cause a phenomenon known as lysis-from-without, whereby the cell is ruptured and infection rendered nonproductive (Delbruck, 1940b). Infection by coliphage T5 results in the inactivation of remaining T5 receptors on the bacterial surface, thus restricting the chances for reinfection (Dunn and Duckworth, 1977).

The adsorption rate is, of course, also dependent on the medium in which the interaction takes place, and on the physiological state of the cells (Delbruck, 1940a). Certain phages adsorb to killed as well as to living bacteria; other phages, such a Lambda, require an energized cell membrane, and adsorb irreversibly only to healthy bacteria (Schwartz, 1976). More subtle variation in adsorption rate may also be significant. The receptor for coliphage Lambda is involved in the uptake of maltose, and the surface density of the receptor depends on the carbon source on which the bacteria are grown. On maltose, the density of receptors is uniformly high, whereas on glucose the concentration of receptors is much lower

(Schwartz, 1976) and highly variable from cell to cell (Howes, 1965; Ryter *et al.*, 1975). These effects are due to the substrate-dependent rate of induction of synthesis of the receptor (Ryter *et al.*, 1975).

2.1.3. *Vegetative Growth and Lysis*

Subsequent to the adsorption of a virulent phage and the penetration of its genetic material, the metabolic machinery of the bacterium comes under the control of the phage genome; to a greater or lesser extent, the bacterium is converted into a "factory" for the production of progeny phage. The duration of the period extending from adsorption to lysis of the cell is referred to as the latent period, and the number of phage progeny released upon lysis is referred to as the burst size.

These parameters can be estimated using the one-step growth experiment devised by Ellis and Delbruck (1939). At time zero, known densities of bacteria and phage are mixed; as with the adsorption rate experiment, bacteria are in excess to avoid the complications of multiple infections. After several minutes, the proportion of phage that have irreversibly adsorbed is determined (preferably near 100%), and the culture is diluted to prevent any further adsorption. This culture is then assayed at frequent intervals for the concentration of plaque-forming units (infected bacteria plus remaining free phage), which should remain unchanged over the duration of the latent period. After an elapsed time equivalent to the latent period, the first infected cells begin to lyse, releasing progeny phage and increasing the concentration of plaque-forming units. During the rise period, all infected cells eventually burst, and a new plateau is reached for the concentration of plaque-forming units, which remains constant because dilution has effectively stopped further phage adsorption and replication. The ratio of the concentration of plaque-forming units after the rise period to their concentration immediately after dilution is the average burst size; this ratio must be corrected for the fraction of phage that were not adsorbed.

Although the intracellular dynamics of phage growth during the latent period can be viewed as a "black box" from the perspective of population dynamics, it is frequently divided into two phases. Prior to the eclipse, an infected bacterium that is lysed artificially (e.g., with chloroform) contains no infective phage; subsequent to the eclipse, but still prior to natural lysis, an artificially lysed bacterium contains increasing numbers of phage progeny. This discontinuity occurs because phage are assembled not one-by-one, but rather *en masse* from precursor subunits.

While adsorption for some phage can occur even on dead bacteria, this would not seem to be the case for vegetative growth. However, coliphage T2 can, under appropriate conditions, actually replicate in and lyse otherwise nonviable cells (T. F. Anderson, 1948). This is because T2 relies almost exclusively on its own genetic information, shutting off all host-controlled macromolecular synthesis within a few minutes after the penetration of its DNA (Koerner and Snustad, 1979). In contrast, vegetative growth by most phage requires a living cell, and host metabolism may continue almost until lysis.

The course of vegetative growth also can be influenced by multiple infections, with the effects dependent on the time elapsed between successive events. For some phage, subsequent infections are usually nonproductive, such that later adsorbed phage are essentially killed. For others, like the T-even coliphages, a phenomenon known as lysis inhibition can occur, in which lysis is delayed (and total burst size increased) by reinfecting the bacteria (Doermann, 1948). It is also frequently possible to demonstrate priority effects, whereby phage of one type partially or completely exclude the production of progeny phage of another type (Delbruck and Luria, 1942). If exclusion is not complete, phage genomes may exhibit recombination, as first shown by Hershey and Rotman (1949).

2.2. Bacterial Defenses and Phage Counterdefenses

2.2.1. *Resistance Mutations in Bacteria*

The rate at which phage irreversibly adsorb to bacteria may be affected by mutations in either the bacteria or the phage. I have already noted that phage adsorption rates can be nearly as high as the maximum rates allowed by Brownian motion, a result undoubtedly due to selection acting on phage to increase the efficiency of their adsorption to specific receptor sites on bacteria. Similarly, bacteria can be selected that have reduced rates of adsorption by particular phage.

Mutant bacteria may be resistant to adsorption by a particular phage for any of several reasons: (1) the structure of the receptor sites may be altered; (2) the exposure of the receptor sites may be altered; (3) the density of the receptor sites may be reduced; or (4) the receptor sites may be lost altogether because of failure in their production or their incorporation into the cell envelope. This fourth class of mutants, altogether lacking particular receptor sites, is especially useful in the identification of receptor moieties through biochemical comparisons of sensitive and resistant cell envelopes (Schwartz, 1980). These mutants also have special evolutionary significance, as they present a challenge that is not readily overcome by mutations in the phage genome.

Because phage receptor sites perform functions of use to the bacte-

rium, their alteration or loss may interfere with bacterial metabolism. I will return to evidence for this point later, and present just two specific examples now. *Escherichia coli* mutants that are resistant to phage Lambda are unable to grow on maltose at low concentrations, although at higher concentrations they are able to grow (Szmelcman and Hofnung, 1975). It appears that Lambda receptors are involved in the active transport of maltose, but that diffusion alone is sufficient for bacterial growth when this substrate is abundant. The coliphage T1 adsorbs to receptor sites that bind and transport iron complexes (Braun and Hantke, 1977), yet E. H. Anderson (1946) demonstrated that some T1-resistant mutants of *E. coli* are auxotrophic for the amino acid tryptophan. This odd result probably derives from the fact that certain T1-resistance mutations map immediately adjacent to the structural genes of the tryptophan operon (Bachmann and Low, 1980). This auxotrophy thus appears to be an indirect consequence of deletion mutations that confer resistance to phage T1.

The rate at which a bacterial strain mutates to resistance to a particular phage can be estimated by means of a fluctuation test developed in the classic paper by Luria and Delbruck (1943). A number of independent cultures of bacteria are grown to some final density, and the entire content of each culture is plated in the presence of excess phage. All bacteria that are sensitive to the phage are killed, whereas any resistant mutants that may be present can grow and are detected. The rate of mutation is estimated from the proportion of cultures that contain zero phage-resistant mutants, according to the Poisson distribution and taking into account the number of bacteria in each culture. It is possible to modify this estimation procedure to make use of the frequency of mutants (rather than just their presence or absence), but this requires an additional assumption concerning the relative growth rates of resistant and sensitive cells.

The phenotypic expression of phage resistance may require one or more generations after the original mutational event, as preexisting phage receptors are diluted sufficiently to allow the survival of progeny in the presence of excess phage (Kubitschek, 1970). The detection of these "latent" mutants requires that the excess phage be added only after plated bacteria have had several generations to grow.

In principle, use of these procedures could result in the isolation not only of mutants that are resistant to phage adsorption, but also of mutants that survive by virtue of their inability to support vegetative growth of the phage. In practice, however, almost all mutants isolated in this way are resistant to phage adsorption. Several factors may account for this. First, for many phage the adsorption process itself is lethal to the

bacterium (Duckworth, 1970); this is especially likely when there are excess phage due to the phenomenon of lysis-from-without. Second, the loss of a bacterial function essential for phage replication is also likely to prevent the growth of the bacteria, hence precluding detection. In contrast, the loss of receptor function, though often deleterious, usually does not completely incapacitate bacteria, probably because most nutrients can be obtained via several routes. Third, most abortive phage infections are due to the acquisition, not the loss, of specific host gene functions. Such events are more likely to occur by transfer of genetic material than by spontaneous mutation.

Rates of mutation to phage resistance can vary considerably, depending on the particular bacterium and phage: as high as 10^{-3} per cell generation for phage that adsorb to the antigenic determinants of phase variation in *Salmonella* and *Shigella* (E. S. Anderson, 1957; Barksdale and Arden, 1974), but more typically on the order of 10^{-7} or less (Demerec and Fano, 1945). Because different phage often share receptor sites (or some component of the receptors), selection for resistance to one phage often results in resistance to other phage. For example, nearly all mutants of *E. coli* B that are resistant to phage T3 are resistant to phage T4, while about one-half of these mutants are also resistant to phage T7 (Demerec and Fano, 1945). T3 and T7 are closely related on morphological and other grounds, but are unrelated to T4 (Bradley, 1967; Kruger and Schroeder, 1981); all three adsorb to components of the lipopolysaccharide core (Wright *et al.*, 1980). In contrast, resistance to these phage is independent of resistance to phage T5, which adsorbs to a proteinaceous receptor.

There is one serious limitation to the standard procedure of isolating phage-resistant mutants by plating bacteria in the presence of excess phage. Such a procedure detects only those mutants that are completely resistant to the selecting phage, whereas bacterial mutants that are partially resistant (i.e., with an adsorption rate parameter that is reduced, but still greater than zero) are killed by the excess phage. It would seem logical to reduce the concentration of phage on the selective plates in order to isolate partially resistant mutants (as is done with antibiotics); but phage (unlike antibiotics) replicate on the sensitive bacteria, thereby defeating this approach. Thus, the standard procedure focuses on mutations of extreme phenotypic effect, and misses mutations that are more subtle but also of ecological and evolutionary significance. There have been a few studies of mutations conferring partial resistance. Lenski (1984a) demonstrated that mutations in *E. coli* B that confer complete resistance to phage T4 also confer partial resistance to phage T2, as indicated by a reduced adsorption rate. Some bacteria produce polysaccharide capsules

that render them partially resistant to certain phage (Wilkinson, 1958; Paynter and Bungay, 1970); the expression of this trait depends on both the bacterial genotype and the culture medium.

2.2.2. *Host-Range Mutations in Phage*

Just as one can select mutant bacteria that are resistant to adsorption by a particular phage, it is also often possible to select mutant phage that are able to infect these resistant bacteria. Such phage are termed host-range mutants. The rate of host-range mutation can be estimated by procedures analogous to those used for estimating rates of bacterial mutation to resistance, except that host-range mutants are detected by plaque formation on lawns of resistant bacteria (Luria, 1945; Hershey, 1946).

Host-range mutants may differ from wild-type phage in two ways: (1) an altered specificity, whereby the configuration of the adsorption organelle of the phage is modified so as to permit the phage to bind to the surface of resistant bacteria; or (2) a reduced selectivity, whereby the threshold for conformational changes that cause irreversible binding is lowered so as to permit the genetic material of the phage to be released more readily into resistant bacteria. In support of the first mechanism, it is possible to substitute the tail fibers of T4 with those of T2 and thereby give resultant T4 the host range of T2 (Wright *et al.*, 1980). In support of the second mechanism, Crawford and Goldberg (1977) isolated host-range mutants of phage T4 that differed not in their tail fibers, but in the baseplate, whose expansion permits subsequent penetration of the phage's genetic material into the host.

More generally, it is noteworthy that most host-range phage mutants are capable of growth not only on bacteria resitance to wild-type phage, but also on wild-type bacteria (Luria, 1945; Chao *et al.*, 1977), and that various host-range mutants can usually be ordered in terms of increasing inclusivity (Hofnung *et al.*, 1976; Manning and Reeves, 1978; Schwartz, 1980). That is, most host-range mutants can be said to have an extended (not just a modified) host range. This seems more consistent with the reduced selectivity mechanism than with the altered specificity mechanism. Added support comes from the observation that many host-range mutants are relatively unstable, spontaneously ejecting their genetic material (and hence losing their infectivity) even in the absence of suitable hosts (Schwartz, 1980; Lenski and Levin, 1985). Thus, it appears that most host-range mutations generate phage with reduced selectivity, binding reversibly to the same basic receptor moiety, but "trigger happy" with respect to the irreversible events in the phage adsorption process (Schwartz, 1980).

As noted, many phage-resistant bacterial mutants have altogether lost particular receptor sites, not just changed their configuration, exposure, or density. The synthesis or incorporation of the receptor moiety may be blocked by any number of deletions, insertions, or point mutations; in short, even mutations that result in the loss of an existing gene function suffice to produce resistant bacteria. In contrast, very specific changes in the functions of one or more phage components would be necessary to generate host-range mutants capable of adsorbing to entirely different receptor sites on such resistant bacteria. Thus, "There can exist two broad classes of phage-resistant bacterial mutants: those for which one can select host-range phage mutants and those for which one cannot select host-range phage mutants" (Lenski, 1984b).

The most elegant demonstration of this fundamental asymmetry comes from the work of Hofnung *et al.* (1976) on phage Lambda and *E. coli* K12. These researchers generated a large set of resistant bacteria using a mutagen that causes single base substitutions. Corresponding host-range phage mutants could be isolated for only a fraction of the resistant clones. Bacterial clones were then tested for the responsiveness of their resistance mutations to nonsense suppressors, genes borne by certain laboratory vectors that allow the bacterial cell to translate a nonsense (i.e., stop) codon into a specific amino acid (Lewin, 1974). Those bacterial clones for which Hofnung and co-workers could isolate corresponding host-range mutants did not respond to nonsense repression, indicating that resistance was the result of missense mutations. In contrast, those bacterial mutants for which they could not isolate corresponding host-range mutants were responsive to nonsense repression, indicating that resistance was the result of nonsense mutations. Missense mutations yield an altered amino acid sequence in the proteinaceous phage receptor, whereas nonsense mutations yield a stop codon and consequently a complete loss of functional receptor sites. Host-range mutants exist only to counter the former. These results are summarized schematically in Fig. 1.

2.2.3. *Restriction and Other Immunities*

Resistance to adsorption is but one kind of bacterial defense against phage infection. A second kind of defense can be termed immunity, and differs from most resistance in several important ways: (1) immunity is manifest intracellularly, rather than on the cell surface; (2) immunity results in the death of the phage, rather than its rejection by the cell; (3) immunity results from the action of a specific gene product, rather than the inactivation of a gene coding for the receptor moiety; and (4) immu-

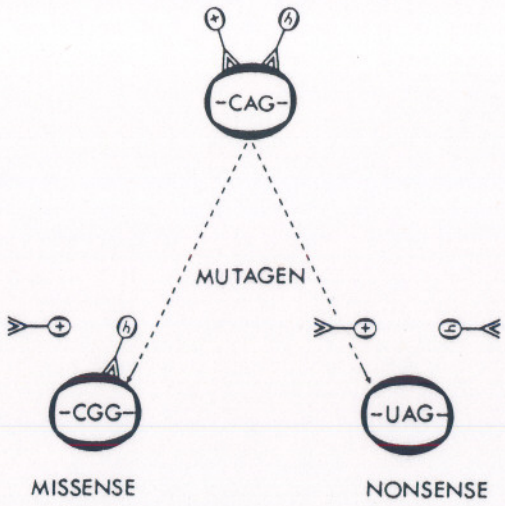

Figure 1. Schematic representation of the results of Hofnung *et al.* (1976). The plus symbol indicates a wild-type phage, while h indicates a host-range mutant. The three hypothetical codons correspond to a bacterial gene encoding a phage receptor, and are wild-type, missense mutant, and nonsense mutant, respectively.

nity is often conferred by the acquisition of an extrachromosomal element, rather than by a chromosomal mutation.

Restriction–modification systems represent one important class of bacterial immunity (Arber and Linn, 1969; Meselson *et al.*, 1972). Through the action of restriction endonucleases, phage DNA may be recognized as foreign and destroyed by the cleaving action of these enzymes. Corresponding modification enzymes protect the bacterial DNA by methylating nucleotide base sequences vulnerable to the restriction enzymes. However, there is some small probability (e.g., 10^{-4}) that the DNA of an infecting phage is accidentally modified. If so, all of the progeny phage are also modified, and after their lytic release they can infect that bacterial strain with full efficiency (Luria, 1953). These progeny are not protected, however, against restriction enzymes borne by other bacterial strains that differ in their specificity.

Restriction and modification enzymes may be encoded by chromosomal, prophage, or plasmid genes. It seems reasonable to postulate that these enzymes evolved as a defense against phage infection (Levin and Lenski, 1985), although they may also function in site-specific repair and recombination (Meselson *et al.*, 1972). Whatever their original function, restriction blocks the vegetative growth of phage, thereby permitting survival of a bacterium. Not surprisingly, many phage possess mechanisms that permit them to avoid the restriction immunity of bacterial hosts (Kruger and Bickle, 1983). For example, DNA of the T-even coliphage

contains the base hydromethylcytosine in place of the normal cytosine, making it resistant to many restriction enzymes. This unusual base is also usually glucosylated, which protects it against the action of other bacterial enzymes. Phage T3 and T7, in contrast, avoid restriction and modification by the early expression of gene products that actively interfere with the host enzymes.

A second class of immunity is that of lysogenic bacteria to reinfection by temperate phage of the same type as the prophage. This immunity is also related to the specific action of a protein, the repressor, which binds to sites on the phage genome and thereby prevents transcription of those genes whose products lead to the lytic destruction of the bacterium (Ptashne *et al.*, 1980).

A third class of immunity derives from a variety of extrachromosomal genetic elements that result in abortive infections by certain phage (Duckworth *et al.*, 1981). For example, the prophage Lambda interferes with the vegetative growth of certain genotypes of the T-even phage, while carriage of an F plasmid results in nonproductive infections by phage T7. In some cases, such immunities may be of adaptive value to the bacteria (and hence also to semiautonomous elements that impart these effects), whereas in other cases the abortive phage infections nevertheless result in death for the infected bacteria. For example, Stone *et al.* (1983) produced a recombinant plasmid containing a portion of the T7 genome that rendered infections by whole T7 viruses nonproductive. This effect apparently derived from the premature expression of a gene that resulted in the lysis of bacteria prior to encapsulation of the replicated phage genomes.

3. Mathematical Models of the Interaction

There are a number of references dealing with various aspects of population modeling. Levins (1966) presents an overview of the strengths and weaknesses of three strategies (generality, realism, precision) in model building. May (1974) provides an extensive mathematical treatment of key issues in species interactions and community structure. May and Anderson (1983) discuss general epidemiological and population genetic models of host–parasite coevolution, while Levin and Lenski (1983) informally treat the coevolution of bacteria and their parasites (including plasmids and temperate and virulent phage). Williams (1972, 1980) and Bull and Slater (1982) present more general discussions of the modeling of microbial populations and interactions. Kubitschek (1970) summarizes some basic chemostat theory.

3.1. Variations on Lotka and Volterra

The most familiar mathematical model used to describe the dynamic relationship between predators and their prey (or parasites and their hosts) is that derived independently by Lotka (1925) and Volterra (1926). According to this model, the dynamics of predator and prey can be described by just four parameters: (1) the rate of growth of the prey population, which is assumed to be constant; (2) the rate of loss in the prey population, which is assumed to be an increasing linear function of the density of the predator population; (3) the rate of growth of the predator population, which is assumed to be an increasing linear function of the density of the prey population; and (4) the rate of loss in the predator population, which is assumed to be constant.

Although it is often criticized for its unrealistic assumptions, the Lotka–Volterra model provides a useful starting point for the development of a more realistic model of the interaction between bacteria and virulent phage. Models developed by Campbell (1961) and Levin *et al.* (1977) differ from the Lotka–Volterra model in two important ways. First, they include some form of density limitation acting on the prey population in the absence of the predator. Second, they include a time lag between the act of predation and the resulting increase in the predator population. A simple model which incorporates these additional factors is presented below.

3.1.1. Dynamic Model of Bacteria and Virulent Phage

Consider an open habitat (like a chemostat) that contains a population of virulent phage, a population of sensitive bacteria, and a potentially limiting bacterial resource. The habitat is liquid and thoroughly mixed, such that phage, bacteria, and resources encounter one another at random. The resource has a concentration C_0 (μg/ml) as it flows into the habitat at a rate ω (turnovers/hr). Uninfected bacteria, infected bacteria, free phage, and unutilized resource are washed out of the habitat at this same rate.

Uninfected bacteria multiply via binary fission at a per capita rate that is a hyperbolic function of the resource concentration in the habitat. The maximum specific growth rate is ψ (hr^{-1}), and K (μg/ml) is the resource concentration at which the bacteria grow at half this maximum rate. Each replication of a bacterium uses up ϵ (μg) of the resource.

Phage encounter and irreversibly adsorb to uninfected bacteria at a per capita rate that is a linear function of the bacterial density. The adsorption constant δ (ml/hr) corresponds to the "search and attack" efficiency of the phage. Each infection is lethal to a bacterium, and each yields β phage progeny after a latent period of τ (hr).

The following differential equations relate the concentrations of resource (C), uninfected bacteria (S), infected bacteria (I), and free phage (P):

$$dC/dt = (C_0 - C)\omega - \epsilon S\psi C/(K + C) \quad (1)$$

$$dS/dt = S\psi C/(K + C) - \delta SP - \omega S \quad (2)$$

$$dI/dt = \delta SP - e^{-\omega\tau}\delta S'P' - \omega I \quad (3)$$

$$dP/dt = \beta e^{-\omega\tau}\delta S'P' - \delta SP - \omega P \quad (4)$$

S' and P' are the concentrations of uninfected bacteria and free phage, respectively, at time $t - \tau$, and $e^{-\omega\tau}$ is the fraction of bacteria infected at time $t - \tau$ that has not washed out of the habitat before lysing.

We can solve for the equilibrium population densities by setting these differential equations equal to zero and performing some relatively simple algebraic manipulations. For the sensitive bacteria (and for the resource), there are actually two distinct equilibria, corresponding to the presence and absence of the virulent phage population. That which obtains in the absence of phage can be termed the resource-limited equilibrium, and is indicated by $\tilde{S}$:

$$\tilde{S} = [C_0 - \omega K/(\psi - \omega)]/\epsilon \quad (5)$$

That which obtains in the presence of phage can be termed the phage-limited equilibrium, denoted by $\hat{S}$:

$$\hat{S} = \omega/[\delta(\beta e^{-\omega\tau} - 1)] \quad (6)$$

Consider the following set of biologically plausible parameters: $\psi = 0.7$ hr^{-1}, $K = 5$ μg/ml, $\epsilon = 2 \times 10^{-6}$ μg, $C_0 = 100$ μg/ml, $\omega = 0.2$ hr^{-1}, $\delta = 1 \times 10^{-7}$ ml/hr, $\beta = 100$, and $\tau = 0.5$ hr. The phage-limited equilibrium density of sensitive bacteria is 2.2×10^4 ml^{-1}, or more than three orders of magnitude below the resource-limited equilibrium of 4.9×10^7 ml^{-1}. Note that the phage-limited equilibrium density of bacteria is in fact independent of the resource concentration, provided only that C_0 is sufficient to support bacterial growth in excess of washout.

The equilibrium density of the phage $\hat{P}$ does, however, depend on the resource concentration:

$$\hat{P} = [\psi\hat{C}/(K + \hat{C}) - \omega]/\delta \quad (7a)$$

$\hat{C}$ is the equilibrium concentration of resources when the sensitive bacteria are phage-limited. If $\hat{S}$ is much less that $\dot{S}$, then phage-limited bacteria are able to utilize only a small fraction of the incoming resources, and $\hat{C}$ is very nearly equal to C_0. If, in addition, C_0 is much greater than K, then the phage-limited bacterial population is effectively growing exponentially at the rate ψ, and Eq. (7a) simplifies:

$$\hat{P} \approx (\psi - \omega)/\delta \tag{7b}$$

These conditions hold for the parameters given above, and yield an equilibrium density for the virulent phage of 5×10^6 ml^{-1}, or roughly two orders of magnitude greater than the equilibrium density for their sensitive bacterial hosts.

Consider the same set of parameters, except now let $\delta = 1 \times 10^{-11}$ ml/hr. This much reduced adsorption rate yields a phage-limited equilibrium density for bacteria of 2.2×10^8 ml^{-1}, which is greater than the resource-limited equilibrium. However, inserting the resource-limited bacterial density into Eq. (4) yields a negative growth rate for the phage population, indicating that these phage cannot become established even when their bacterial hosts are at maximum density. In such cases, Eqs. (6) and (7) are invalid, as there does not exist a phage-limited state.

The model specified by Eqs. (1)–(4) corresponds closely to the basic description of lytic infections presented in Section 2.1. Of course, it contains many assumptions, two of which are as follows: (1) all of the parameters remain constant through time; and (2) there is no variation within the populations. In the next two sections, we will consider the effects of violating these assumptions.

3.1.2. Stability and Complexity

The Lotka–Volterra model is neutrally stable. That is, both predator and prey populations exhibit oscillations of constant frequency and amplitude, with the predator lagging one-quarter phase behind the prey. The phage–bacteria model just presented differs structurally from the Lotka–Volterra model in two respects. First, the rate of growth of the bacteria is a function of a potentially limiting resource. Second, there is a time lag between phage infection and multiplication. The former tends to stabilize the interaction, whereas the latter is destabilizing. The net effect of these opposing forces depends on the specific parameters used in the equations; the model can exhibit stable equilibria, stable oscillations, or oscillations leading to extinction (Levin *et al.*, 1977).

The relative magnitudes of the phage-limited and resource-limited equilibrium densities of bacteria are especially important. If the difference is slight, then the extent to which bacteria overshoot the phage-limited equilibrium is reduced as resource limitation becomes a significant factor, and the interaction will be stabilized (Fig. 2a). On the other hand, if the difference is great, then resource limitation has little effect, and the destabilization that results from the time lag will predominate (Fig. 2b).

It is also of interest to ask how variation in environmental parameters influences the interaction. For example, consider virulent phage and bacteria interacting in a "seasonal" habitat, one that is closed except at occasional intervals when resources are renewed (Stewart and Levin, 1973). In Fig. 2c, the dynamics of virulent phage and bacteria is modeled for the same set of parameters as in Fig. 2a, except that the community is cultured serially, not continuously. That is, instead of a flow rate of 0.2 hr^{-1}, there is a transfer of 1% of the community into a fresh culture every 24 hr. While the interaction in chemostat culture is quite stable, it is not at all stable in serial culture. The virulent phage drive the sensitive bacteria to extinction even before the first transfer; extinction of the phage

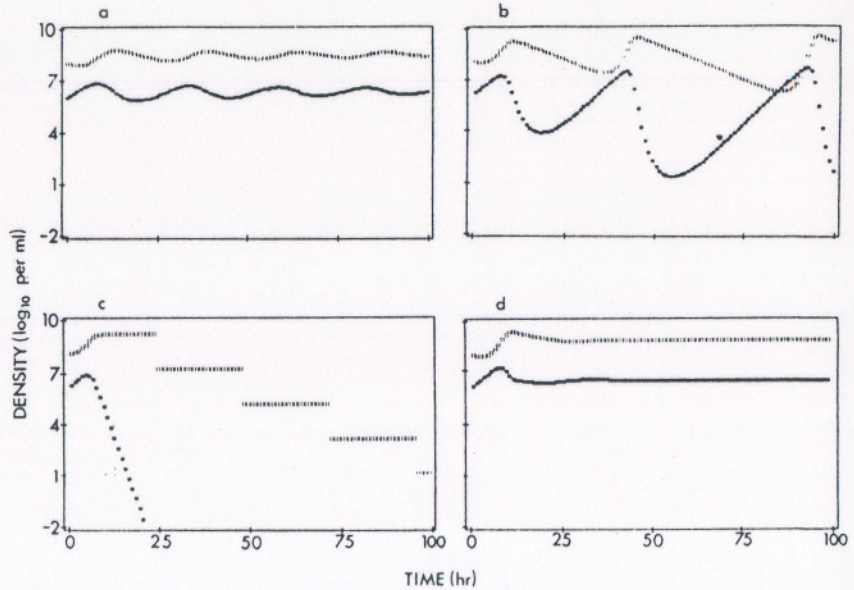

Figure 2. Dynamics of the model of the interaction between bacteria and virulent phage. Lighter symbols indicate free phage; darker symbols indicate uninfected bacteria. Parameter values are as in Section 3.1, except as noted. (a) $\delta = 1 \times 10^{-9}$ ml/hr, $C_0 = 25$ μg/ml. (b) $\delta = 1 \times 10^{-9}$ ml/hr, $C_0 = 100$ μg/ml. (c) As for (a), except in serial culture. (d) As for (b), except with nongenetic heterogeneity in bacterial vulnerability to phage infection.

results from subsequent daily dilutions. This instability occurs in serial transfer because continuous phage mortality due to washout is lacking.

I will present evidence later that many interactions between virulent phage and bacteria are more stable than anticipated from theory. One hypothesis that could account for this stability is the existence of refuges, either spatial or physiological, for sensitive bacteria. The simplest way of modeling a refuge is to allow two states for the bacteria, vulnerable and protected. The probabilities of a daughter cell being in one state or the other are *independent* of the state of the bacterium that is dividing; hence, the variation is not heritable. (The protected state is *not* equivalent to resistance, which is heritable. Resistance will be treated in Section 3.2.) In Fig. 2d, I have graphed the dynamics in chemostat culture using the same parameters as in Fig. 2b, except that each daughter cell now has one chance in ten of being protected from the phage.

It may be surprising that this variation, while greatly stabilizing the interaction, has little effect on the equilibrium densities of bacteria and phage. This can be understood, however, by recognizing that the protected population is *not* self-sustaining. The maximum growth rate for the protected population is 0.7 hr^{-1}, but 90% of the bacteria that are produced each generation are vulnerable to the phage. The remaining 10% are insufficient to offset washout at the rate of 0.2 hr^{-1}. Thus, the protected population is maintained only as a "by-product" of the vulnerable population. The situation could be changed dramatically, however, if the probabilities that any given daughter cell is vulnerable or protected were reversed. No change is implied in the heritability (or rather lack thereof) for the trait; the probabilities are still independent of the state of the generating bacteria. But with 90% of the bacteria produced each generation protected from the phage, the total bacterial population would rapidly approach resource limitation. Nonetheless, the virulent phage population could persist by exploiting the vulnerable population, which in this case would become a non-self-sustaining "by-product" of the protected population.

Smith (1972) provides an excellent discussion of the role of heterogeneity in stabilizing populations, while Alexander (1981) reviews a variety of factors that promote the coexistence of microbial parasites and hosts.

3.2. Evolutionary Considerations

The analysis of adaptive changes in the bacteria and phage populations requires two distinct considerations. First, the model must indicate the rate at which mutants (or recombinants) appear in the community. Thus far, all processes in the model have been assumed to be deterministic. This is reasonable when rates are large relative to the inverse of population sizes, as is likely for most ecological processes. It may not be reasonable, however, for relatively rare events associated with genetic changes. Not only may the appearance of a new genotype be stochastic, but so may be its fate. That is, even if a new genotype has a net selective advantage, there is a certain probability that it will be lost (e.g., due to washout) before replicating. In this chapter, I will not specifically model the origin of new genotypes. Lenski and Levin (1985) discuss the stochastic appearance and fate of mutant phage-resistant bacteria and host-range phage. Levin (1981) models the appearance of recombinant genotypes in bacterial populations.

Second, the model must incorporate the subsequent growth of the new mutant (or recombinant) population and its interaction with other populations. In particular, it is of interest to ask whether the new population can become established, and if so, what are its equilibrium density and its effects on the equilibrium densities of the other populations.

We can easily write a differential equation for the dynamics of a resistant population, using subscript R to differentiate growth parameters from those for sensitive bacteria in Eq. (2). [It is assumed here that the resistance is absolute, although partial resistance can also be modeled (Levin *et al.*, 1977).] We have

$$dR/dt = R\psi_R C/(K_R + C) - \omega R \tag{8}$$

Let us first consider whether a resistant population can invade a chemostat at phage-limited equilibrium. Recall that the concentration of resources in a phage-limited chemostat $\hat{C}$ is greater than the concentration in a resource-limited chemostat. If the growth parameters are identical for sensitive and resistant bacteria, then resistant bacteria can clearly invade as they have the same rate of replication, but fewer losses. In fact, they can increase even if they have a somewhat lower rate of growth, provided only that their rate of growth at resource concentration $\hat{C}$ exceeds the rate of flow through the habitat. In general, the greater the difference is between the phage-limited and resource-limited equilibrium densities of sensitive bacteria, the broader the conditions are for establishment of a resistant population. Resistant bacteria fail to invade only if they are so much less efficient at extracting resources and growing that they cannot offset losses due to washout.

If the resistant populations can invade, it increases until it becomes resource-limited, at which point its growth is exactly offset by losses to washout. If the growth parameters of the resistant bacteria are identical to those of the sensitive bacteria, then its resource-limited equilibrium is identical to that which occurs for the sensitive bacteria in the absence of

phage. In this case, the sensitive population has no growth advantage, but sustains additional losses due to phage infection; it is driven to extinction as a consequence. With the extinction of the sensitive bacteria, the virulent phage population is also driven to extinction, unless host-range mutants arise that can infect the resistant bacteria.

On the other hand, if the resistant bacteria have a lower growth rate than the sensitive bacteria, then their resource-limited equilibrium is also lower. More important, the equilibrium concentration of resource that sustains the growth of the resistant population at a rate just sufficient to offset washout must allow a higher growth rate for the sensitive bacteria. Hence, sensitive bacteria can persist subsequent to the attainment of resource limitation by phage-resistant bacteria. In fact, the evolution of resistant bacteria has no effect at all on the equilibrium density of the phage-limited sensitive population; from Eq. (6), we see that this equilibrium is independent of resource concentration. In contrast, the equilibrium density of the virulent phage population is reduced by the evolution of a resistant bacterial population; from Eq. (7a), we see that this equilibrium is proportional to the growth rate of the sensitive population, and hence depends on the resource concentration. In effect, the resistant bacteria sap some (but not all) of the excess growth (relative to washout) of the sensitive bacteria, which supports the virulent phage.

Wild-type phage can thus persist subsequent to the evolution of resistant bacteria, provided that their sensitive hosts have a growth rate advantage when resources limit the resistant population. Nonetheless, there is strong selection for host range phage mutants subsequent to the evolution of resistant bacteria; a resource-limited bacterial population provides a superabundance of potential hosts. I will not present an equation for the dynamics of a host-range phage population, as notation becomes further complicated and the equations for bacterial dynamics would have to be revised. However, the conditions for coexistence of wild-type and host-range phage populations are similar to those for sensitive and resistant bacteria. If sensitive bacteria have a growth rate advantage over resistant bacteria, *and if* host-range phage mutants exploit the sensitive bacteria less efficiently than wild-type phage, then the two phage genotypes and the two bacterial genotypes can coexist.

4. Laboratory Communities

From the mathematical model of the interaction between bacteria and virulent phage, two fundamental predictions should be emphasized. (1) Virulent phage can limit sensitive bacteria to a density below that set by resources. However, the stability of the interaction may depend on the availability of some refuge for sensitive bacteria, because in the absence of a refuge the interaction may produce oscillations leading to extinction. (2) Mutations conferring phage resistance in the bacterial population or extended host range in the phage population are generally favored. However, if phage-resistant bacteria are at a competitive disadvantage for resources, then sensitive bacteria can persist and thereby maintain virulent phage, even if no host-range phage mutants evolve.

4.1. Ecological Dynamics

It is possible to demonstrate an effect of virulent phage on bacteria in continuous culture by comparing bacterial densities either before and after the addition of virulent phage, or before and after the evolution of resistant bacteria. In principle, the former appraoch is preferable, since resistant bacteria may differ not only in their sensitivity to phage, but also in the efficiency with which they exploit their resources. In practice, however, the effects of virulent phage on bacterial densities in continuous culture are usually so great as to make this complication insignificant.

Data from a number of studies on the dynamics of bacteria and virulent phage in chemostat culture are summarized in Table I. The table includes "order-of-magnitude" approximations for (1) resource-limited equilibrium densities for bacteria $\hat{S}$; (2) phage-limited equilibrium densities for bacteria $\hat{S}$; and (3) equilibrium densities for virulent phage prior to the evolution of resistant bacteria $\hat{P}$. With one exception, *these studies demonstrate a profound effect of virulent phage on the density of sensitive bacteria in chemostat culture.* Phage-limited densities were two to four orders of magnitude below resource-limited densities obtained in the

Table I. Summary of Chemostat Experiments on Virulent Phage and Bacteria[a]

Reference[b]	Phage	$\hat{S}$	$\hat{S}$	$\hat{P}$
Paynter and Bungay (1969, Fig. 3)	T2	9	7	9
Horne (1970, Fig. 1A)	T3	8	4	6
Levin *et al.* (1977, Fig. 5A)	T2	8	4	6
Chao *et al.* (1977, Fig. 3)	T7	8	4	6
Lenski (1984a, Fig. 1)	T2	8	4	6
Lerner (1984, Fig. 1)	MS2	9	9	8
Lenski and Levin (1985, Fig. 1)	T4	8	4	6

[a] $\hat{S}$ is the equilibrium density of resource-limited bacteria, $\hat{S}$ is the equilibrium density of phage-limited bacteria, and $\hat{P}$ is the equilibrium density of phage. All densities are expressed as $\log_{10}$ per ml.

[b] Approximate densities were obtained from the figure that is indicated for each reference. These values are necessarily rough because of the often pronounced variation even between successive samples. The various studies differed somewhat in culture conditions. Most notably, Paynter and Bungay (1969) used broth at 30°C, whereas all others used minimal media at 37°C. Rates of flow through the chemostats ranged from 0.04 to 0.3 turnover/hr.

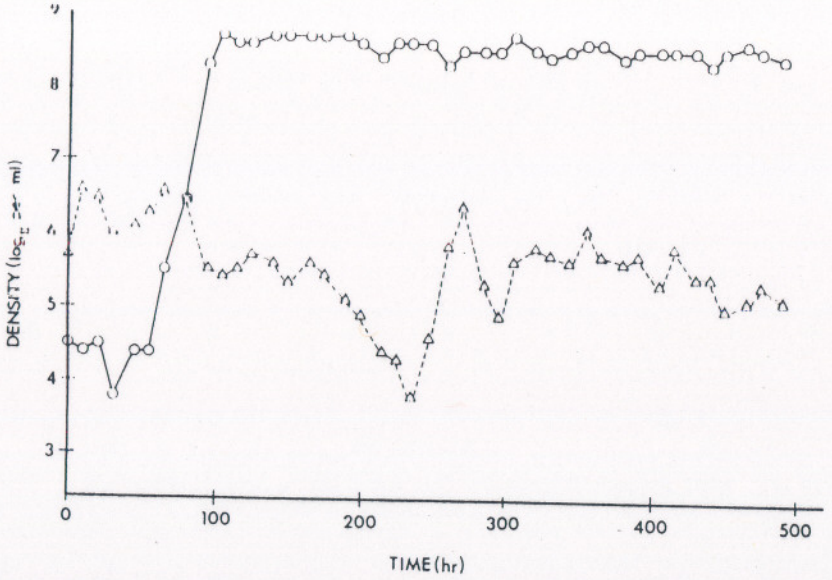

Figure 3. Dynamics of the interaction between *E. coli* B and virulent phage T4 in chemostat culture. Solid line gives bacterial density; dashed line gives phage density. [From Lenski and Levin (1985, Fig. 1), with permission of *The American Naturalist*, © 1985 by the University of Chicago.]

same medium either prior to the addition of virulent phage or subsequent to the evolution of resistant bacteria. This difference can be seen in Fig. 3.

In three of these studies, the authors obtained independent estimates of adsorption rate δ, burst size β, latent period τ, maximum bacterial growth rate ψ, and flow rate ω. By incorporating these parameters into Eqs. (6) and (7b), it is possible to estimate the equilibrium densities for phage-limited sensitive bacteria and for virulent phage. The parameter estimates and corresponding predicted equilibria are given in Table II. Equilibria predicted by Levin *et al.* (1977) working with *E. coli* B and phage T2, and by Lenski and Levin (1985) using B and T4, are within an order of magnitude of those observed (Table I). This degree of accuracy lends support to the general validity of the model, especially since the range of plausible equilibrium densities covers many orders of magnitude.

Very different results were observed by Lerner (1984) for the male-specific phage MS2 and a plasmid-bearing strain of *E. coli* K12 that is derepressed (i.e., fully expressed) for conjugative pili synthesis. The parameters estimated for this interaction yield a predicted phage-limited equilibrium of less than 10^7 ml^{-1} (Table II), whereas the observed density

of bacteria was about 10^9 ml^{-1}, indistinguishable from resource limitation (Table I). Moreover, the observed phage density was about three orders of magnitude below the anticipated equilibrium. These results indicate that this phage is much less effective at exploiting the bacteria than predicted from the model. But it is clear that phage were "eating" something in the chemostats; they persisted despite washout, and mutants resistant to the phage increased in frequency in the bacterial population.

It is likely that only a fraction of the derepressed bacteria were actually vulnerable to MS2 at any given time, a scenario that would be consistent with the very low adsorption rate. Although this hypothesis was not explicitly tested, it is supported by one difference between male-specific and most other phage. Whereas receptor sites for most phage are present at high multiplicities per bacterium (e.g., Schwartz, 1976), only one or a few conjugative pili are present on an individual cell. In fact, Brinton and Beer (1967) report that typically 50–90% of the bacteria in a fully expressed culture are actually free of any pili; adsorption by male-specific phage is necessarily restricted to those individuals that happen to have pili. These considerations, in conjunction with Lerner's (1984) chemostat observations, suggest a very high degree of nongenetic heterogeneity among individual bacteria in their vulnerability to male-specific phage. As discussed in Section 3.1.2, this variation could provide a refuge, and thereby cause a discrepancy between observed densities and those predicted by a model in which it is implicitly assumed that all individuals are identical.

According to the mathematical model, the stabilizing effect of bacterial resources on the interaction between sensitive bacteria and virulent phage is apparent only when bacteria are near their resource-limited equi-

Table II. Estimates of Parameters and Predicted Equilibrium Densities for Virulent Phage and Sensitive Bacteria[a]

Estimate	Levin *et al.* (1977) T2	Lerner (1984) MS2	Lenski and Levin (1985) T4
ψ (hr^{-1})	0.7	0.9	0.7
ω (hr^{-1})	0.1	0.2	0.3
δ (ml/hr)	6×10^{-8}	1×10^{-11}	3×10^{-7}
β	100	10,000	80
τ (hr)	0.5	0.8	0.6
$\hat{S}$	4.2	6.4	4.2
$\hat{P}$	7.0	10.9	6.1

[a]Predicted equilibria may be compared with observed densities presented in Table I. Symbols are defined in the text. Densities are expressed as $\log_{10}$ per ml.

librium. But the summary presented in Table I indicates that phage-limited bacterial densities are generally orders of magnitude below resource limitation, suggesting that the dynamics of the interaction could often be unstable. A perusal of the figures cited in Table I indicates that the interactions between virulent phage and sensitive bacteria are less stable than the interactions between bacteria and their resources. Rather than try to quantify this claim, I will let Fig. 3 be illustrative of this general tendency. Note in particular how much more variable are the phage densities than the bacterial densities, especially after the bacteria have attained resource limitation by evolving resistance.

Despite the apparent instability of the interaction between bacteria and virulent phage, reports of extinctions are few. Levin *et al.* (1977) observed that the model predicted oscillations of increasing amplitude leading to eventual extinction when the parameters were in the range estimated for *E. coli* B and virulent phage T2. In fact, oscillations tended to become less, not more, pronounced over the course of their experiments, and no extinctions were observed. Horne (1970) reports the coexistence of phage T3 and *E. coli* B for 80 weeks, and the coexistence of T4 and B for 52 weeks. Of the studies cited in Table I, only Lerner (1984) and Lenski and Levin (1985) report extinctions. Lerner (1984) observed extinctions of MS2 on plasmid-bearing strains of *E. coli* K12 that were *repressed* for conjugative pili synthesis. These extinctions, however, are perhaps better described as failures of the phage to become established. Phage inoculated into chemostats containing the repressed bacteria generally disappeared at a rate equal to the rate of washout. Lenski and Levin (1985) reported three extinctions, of both bacteria and phage, in ten replicate chemostats containing *E. coli* B and T4. Lenski and Levin (1985) also observed extinctions of virulent phage T5 in chemostats with *E. coli* B. The T5 extinctions, however, were subsequent to the evolution of resistant bacteria, and will be discussed later.

There is no continuous source of mortality to a phage population in serial culture comparable to washout in chemostat culture. Hence, the possibilities of stable coexistence would seem more limited, as virulent phage may reach high densities for a sufficient length of time to encounter and destroy all sensitive bacteria, as shown in Fig. 2c. It is certainly the conventional wisdom that in preparing phage lysates, sensitive bacteria are driven extinct as the culture is cleared, even though the culture may eventually become turbid with resistant bacteria. R. E. Lenski, B. R. Levin, and R. V. Evans (unpublished data) have examined the dynamics of virulent phage and bacteria in two serial transfer habitats. One of the habitats consisted of 10 ml of liquid broth, continuously agitated, from which 0.1 ml (or 1%) was removed daily and placed into 9.9 ml of fresh broth. The other habitat consisted of phage-infected colonies on agar

plates, small fractions (approximately 3%) of which were removed each day and transferred to fresh agar plates. Densities of virulent phage and bacteria in liquid culture were assayed by standard dilution and plating techniques. In surface culture, the persistence of bacteria was documented simply by noting the formation of a visible colony subsequent to transfer; the persistence of phage was documented by transferring a portion of the colony to a lawn of sensitive bacteria and observing lysis if phage were present. In each habitat, we examined the interactions of an *E. coli* K12 host and six different virulent phage. For each phage, we ran three replicates in the liquid habitat and 50 replicates in the surface habitat. (The surface replicates were extremely simple, as all 50 communities could be maintained on one plate.)

Results in the surface habitat are best summarized by noting that the six virulent phage used in these experiments fell into two groups producing qualitatively different outcomes. Populations of phage T5 and T7 went extinct at very high rates, more than 90% of T5 and almost 40% of T7 after ten transfers. In contrast, *none* of the populations of T2, T4, T6, or a virulent mutant of Lambda were extinct after 12–20 transfers. The latter phage did not even approach extinction, but maintained high densities from transfer to transfer, indicating the persistence of bacteria that they could exploit. It is probably significant that these two groups also differ strikingly in the appearance of plaques and of infected colonies. Both T5 and T7 produce very large plaques, and infected colonies are translucent. In contrast, the other four phage produce small plaques, and infected colonies remain opaque but have "nibbled" outer edges. Thus, it appears that persistence of phage T2, T4, and T6 and virulent Lambda was due, at least in part, to the existence of spatial refuges for sensitive bacteria in the physically structured surface habitat.

In contrast, there would seem to be no opportunity for spatial refuges in liquid serial culture; vessels were replaced daily and only liquid was transferred. Yet for many of the phage, stable communities were maintained even in this habitat. In fact, only phage T5 behaved according to expectation. In all three replicates, T5 rapidly attained high densities; with subsequent dilutions, phage went extinct, although resistant bacteria flourished. A similar extinction was observed for one virulent Lambda population, but in two other replicates, phage persisted at high densities for 25 transfers, as seen in Fig. 4. Populations of phage T2, T4, T6, and T7 were also generally stable in liquid serial culture.

Although spatial refuges are unlikely to be important in liquid serial culture, several other factors could stabilize these interactions. One possibility is that some genetically sensitive cells are physiologically insensitive. This possibility was already raised in conjunction with the surprising results obtained in continuous culture with the male-specific

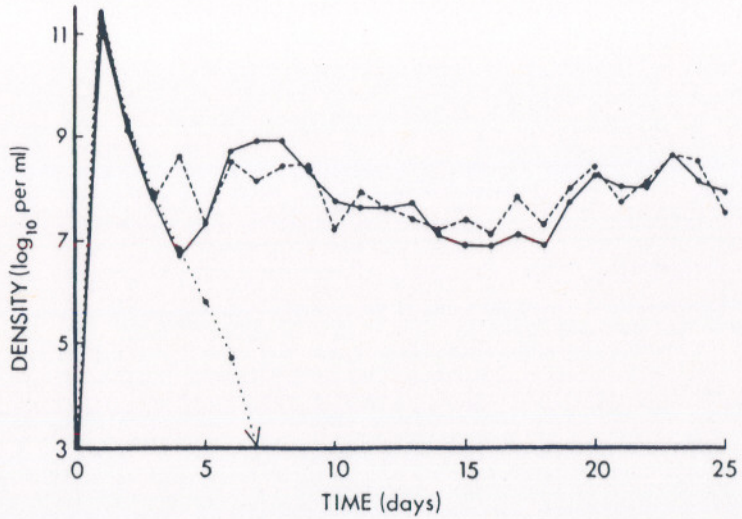

Figure 4. Dynamics of the interaction between *E. coli* K12 and a virulent mutant of phage Lambda in liquid serial culture. Separate lines correspond to three replicate experiments. Only phage population densities are shown; these were obtained just prior to daily transfers [R. E. Lenski, B. R. Levin, and R. V. Evans (unpublished data)].

phage MS2. Physiological protection against phage adsorption, though less dramatic, has also been demonstrated for other phage. Recall, for example, that phage Lambda adsorbs to receptors involved in the uptake of maltose, and that there can be considerable variation among bacteria in the number of these receptors, especially when bacteria are grown on other carbon sources. Physiological refuges may also arise as the consequence of starvation of bacteria (Delbruck, 1940a), depletion of a factor in the medium required for phage adsorption (Luria and Steiner, 1954), or bacterial clumping (Paynter and Bungay, 1970). Physiological refuges may even arise as a consequence of the phage–bacteria interaction; endolysin released by T7-infected bacteria can strip T7 receptors from the surface of surviving sensitive bacteria (Li *et al.*, 1961).

A second possibility is that genetically resistant bacteria either renew or protect the genetically sensitive population. Renewal could come about through back-mutation, although resistance may often arise by deletion and other irreversible events. Protection could occur if phage bind reversibly to (but do not infect) resistant bacteria. This would be equivalent to "hiding" a few sensitive bacteria among the many resistant cells. A test of this possibility was carried out by Stent and Wollman (1972) in their original demonstration of the two-step nature of phage

adsorption (see Section 2.1.2). They found no effect of a high density of resistant bacteria on the rate of adsorption of phage T4 to sensitive cells.

A final possibility is that phage persist by continuously evolving host-range mutants (Rodin and Ratner, 1983). However, the experiments presented above do not support this hypothesis. For example, although bacteria resistant to Lambda evolved, no host-range mutants arose. [Host-range mutants of Lambda can be found that infect certain classes of resistant bacteria, but not all (Hofnung *et al.*, 1976).] In Section 4.2, I discuss more generally the limits to bacterial resistance and phage host range.

4.2. Evolutionary Change

A mutation that confers resistance to a virulent phage can increase the density of bacteria in chemostat culture by several orders of magnitude, as seen in the comparison of phage-limited and resource-limited bacterial densities (Table I). Accompanying this dramatic increase in bacterial density is a dramatic decrease in the availability of unused resource in the habitat. For example, Chao *et al.* (1977) found that the concentration of glucose in a phage-limited chemostat was indistinguishable from the 100 μg/ml concentration in the reservoir, while the concentration of free glucose in a resource-limited chemostat was only about 5 μg/ml. By the same token, a host-range mutation in a virulent phage population can cause a resource-limited chemostat to become phage-limited.

In all of the studies cited in Table I, resistant bacterial mutants were observed in at least some of the longer running experiments. It may take only tens of hours for *E. coli* B that are resistant to phage T4 to arise (Horne, 1970) or several hundred hours for the appearance of B mutants resistant to T2 (Levin *et al.*, 1977). The time required for this evolution depends on several investigator-controlled variables, as well as certain innate characteristics of the particular interaction. For example, Horne (1970) reports resource-limited communities of T4-resistant bacteria after only about 20–40 hr, whereas Lenski and Levin (1985) did not observe this state until about 80–140 hr (see Fig. 3). Two differences in experimental setup probably account for this difference. First, Horne (1970) began his experiments by adding the phage to a resource-limited population of sensitive bacteria, whereas Lenski and Levin (1985) began their experiments with bacteria at a density near their phage-limited equilibrium, several orders of magnitude lower. In the former case, resistant mutants probably were present at the start of the experiment (quite possibly tens or hundreds/ml), whereas they had to arise *de novo* in the latter case. Second, the flow rate in Horne's (1970) chemostats was only about

0.04 hr^{-1}, whereas it was about 0.3 hr^{-1} in the chemostats of Lenski and Levin (1985). A resistant mutant experiencing mortality due only to washout (and not to phage infection) requires only 1.06 hr to double at the lower flow rate, but 1.76 hr at the higher rate, assuming an intrinsic doubling time of 1 hr.

The time required for the evolution of resistant bacteria also depends, of course, on biological features of the interacting bacteria and phage. Resistance to most phage arises via a single genetic event; for example, the mutation conferring resistance to phage T4 by *E. coli* B occurs at a frequency of about 10^{-7} per cell generation. Lenski and Levin (1985) not only found reasonable agreement between observed and predicted equilibrium densities for B and T4, but also between observed and predicted times to the attainment of a phage-resistant, resource-limited bacterial population.

Resistance to virulent phage T2 is much more difficult to obtain. However, it was noted by Lenski (1984a) that T2 resistance by *E. coli* B evolves in chemostats more rapidly than anticipated with the rate of mutation estimated by the fluctuation test (see Section 2.2.1). This discrepancy occurs because only selection in chemostats can enrich mutants that are just partially resistant to phage. Lenski (1984a) demonstrated that resistance to T2 can evolve not only by a single rare mutation, but also by a pair of common mutations, one of which is the same mutation conferring resistance to phage T4. These T4-resistant intermediates adsorb T2 at only about half the rate of T4-sensitive bacteria, and mutate to complete T2 resistance at a rate about two orders of magnitude higher.

Although resistant bacteria appeared in at least some of the longer running experiments in all of these studies, this was not the case for host-range phage mutants. Host-range mutants were observed by Chao *et al.* (1977) for phage T7 and by Lenski and Levin (1985) for phage T2 and T7, but Lenski and Levin (1985) failed to detect host-range mutants of T4 or T5. Host-range phage mutants probably also appeared in the study by Horne (1970) with T3, judging by the time required for resistant bacteria to reach resource-limited density, but they were not specifically mentioned. Where host-range phage mutants evolved, higher order resistant bacteria also evolved, and *the most highly evolved communities were dominated by resource-limited bacterial genotypes that were resistant to all co-occurrring phage genotypes* (Chao *et al.*, 1977; Lenski and Levin, 1985). Despite this, only phage T5 was observed to go extinct with the evolution of resistant bacteria (Lenski and Levin, 1985). This general result is again illustrated in Fig. 3. Note that although the bacteria evolved resistance and there were no corresponding host-range mutations, the phage persisted indefinitely.

In fact, virulent phage may persist subsequent to the evolution of

resistant bacteria, without extending their host range, by continuing to exploit sensitive bacteria that themselves persist due to superiority in competition for resources. However, these basic chemostat experiments do not exclude the possibility that phage are actually capable of growing on the "final" resistant bacterial genotype, but with such meager infectiousness that plaques are not observed. Verification of the former hypothesis thus requires an independent demonstration of one or more of the following: (1) in the presence of phage, sensitive bacteria persist subsequent to the attainment of the resource-limited equilibrium by resistant bacteria; (2) in the absence of phage, sensitive bacteria have a growth rate advantage over resistant bacteria at the resource-limited equilibrium; and (3) no phage genotype has the capacity to reproduce when only resistant bacteria are present.

Although the first of these tests is the most direct, it is also the most difficult. This is because sensitive bacteria are anticipated to be a small minority that cannot be directly selected. Recall from theoretical considerations presented in Section 3 that the phage-limited equilibrium density of sensitive bacteria is independent of the concentration of primary resource, and consequently is not affected by the presence of competitively inferior, but resource-limited, resistant bacteria. This phage-limited equilibrium is typically orders of magnitude lower than the equilibrium set by resources (Table I). And while it is possible to detect a minority of resistant cells by plating in the presence of phage, there is no analogous procedure that allows detection of a sensitive minority. Thus, explicitly demonstrating the persistence of sensitive cells subsequent to the evolution of the resistant population is like looking for a "needle in a haystack." A much cleverer approach was used by Chao *et al.* (1977). They initiated chemostat cultures with virulent phage T7 and both sensitive and resistant *E. coli* B strains, the sensitive carrying a selectable fermentation marker. A minority population of sensitive bacteria was thereby directly shown to persist even in the presence of phage.

Chao *et al.* (1977) also allowed the sensitive and resistant strains to compete in the absence of phage T7. As hypothesized, the sensitive bacteria prevailed, a result shown to be independent of the fermentation marker. Lenski and Levin (1985) found T2-, T4-, and T7-resistant strains to be at a competitive disadvantage with regard to the unselected *E. coli* B strain. The T4-resistant strain declined at a rate of 0.16 hr^{-1} in a chemostat with a flow rate of 0.3 hr^{-1}, indicating a selective disadvantage under these conditions of about 50%. Paynter and Bungay (1969) did not explicitly allow sensitive and resistant bacteria to compete in their chemostats, although they did note that many of the T2-resistant mutants had lower exponential growth rates and longer lag times when introduced into fresh medium.

In contrast, Lenski and Levin (1985) found no measurable competitive disadvantage associated with resistance to phage T5. In fact, T5 resistance has been widely used as a selectively neutral marker in other studies on microbial competition and evolution (Dykhuizen and Hartl, 1983). Recall that only phage T5 was observed to go extinct subsequent to the evolution of resistant bacteria, an outcome entirely consistent with the failure to observe any competitive disadvantage.

Lenski and Levin (1985) also demonstrated that none of the phage T2, T4, T5, and T7 could be supported by corresponding "final" resistant bacterial populations. Furthermore, the maximum rate of host-range mutation consistent with the failure to observe phage multiplication could be back-calculated from the initial size of the phage population. Using this approach, Lenski and Levin (1985) concluded that further host-range extensions, if they exist at all, must occur at rates on the order of 10^{-11} per virus replication or less. Thus, these experiments lend further support to the notion of a fundamental asymmetry in the coevolutionary relationship between bacteria and virulent phage. There exist bacterial mutations conferring resistance to phage for which there are no (or only extremely rare) corresponding host-range mutations. Virulent phage may persist, however, because bacterial resistance often engenders a reduction in competitive ability.

Whereas these studies indicate that a tradeoff between phage resistance and competitive ability occurs frequently in bacteria, it is not known how this tradeoff affects the subsequent course of bacterial evolution. In the absence of phage, will resistant bacteria revert to sensitivity? This seems unlikely to be the general case, since many spontaneous mutations conferring resistance could be the result of deletions and other irreversible events. If not, will the resistant bacteria remain competitively inferior through evolutionary time, or will they find alternative solutions to metabolic limitations?

Just as phage-resistant mutants are often at a competitive disadvantage with regard to wild-type bacteria, so may host-range mutants be at a disadvantage compared to wild-type phage when competing for sensitive hosts. Chao *et al.* (1977) allowed wild-type and host-range phage T7 mutants to compete, and found that the wild-type phage did, in fact, have an advantage in culture with sensitive hosts. Subsequent to the appearance of resistant bacteria, the host-range phage mutants increased, as expected. Lenski and Levin (1985) found that a T7 host-range mutant and one of two T2 host-range mutants survived more poorly than their wild-type counterparts in the presence of bacteria resistant to both. These disadvantages may result from the reduced selectivity of host-range mutants (see Section 2.2.2), which increases the likelihood of errors in adsorption and penetration.

One consequence of such tradeoffs, both in bacteria and phage, is to increase the genetic diversity that can be maintained in these communities, as discussed in Section 3.2. Chao *et al.* (1977) found that at least three bacterial genotypes (wild-type, first-order resistant, and second-order resistant) and two phage genotypes (wild-type and host-range mutants) could coexist stably in a chemostat with glucose as the limiting nutrient.

Malmberg (1977) investigated evolutionary changes in the virulent phage T4 due not just to mutation, but to recombination as well. Malmberg (1977) was able to manipulate the frequency of recombination in the phage population by controlling the multiplicity of phage infection; recall that recombination occurs when multiple phage infect a single bacterium. The phage population size was kept constant over all levels of recombination, and phage were restricted to a single round of adsorption and lytic replication per experimental generation. No evolution took place in the bacteria, because the same strain was cultured anew for each phage generation. The fitness of phage in any experimental treatment was defined as the number of progeny per parental phage per generation. The degree to which epistasis contributed to any increase in fitness was determined by crossing an evolved phage line with a phage line bearing a number of markers and examining the contribution of various regions of the phage genome to the observed change in fitness. Malmberg (1977) found that increases in the fitness of the phage were more rapid at the higher frequency of recombination, and that epistatic gene interactions were more important at the lower frequency of recombination. Malmberg (1977) also partitioned the fitness changes into several components of the phage life cycle. Burst size contributed much more than adsorption rate, a result not surprising given the imposition of a life cycle consisting of discrete (not overlapping) generations. Because bacteria did not coevolve in this experiment, the role of phage recombination in host-range shifts was not investigated.

5. Natural Communities

Can the ecological and evolutionary generalizations obtained from laboratory communities be extended to nature? Such extrapolation is difficult because natural communities contain so many additional complexities that have not been considered in the laboratory. Nonetheless, I will attempt to pursue one generalization that gives rise to reasonably specific predictions. That is, the apparent fundamental asymmetry in the coevolutionary potential of bacteria and virulent phage implies that most natural communities of bacteria (or at least coliform bacteria, on which the

laboratory studies have focused) should consist of dominant clones resistant to all co-occurring phage. Virulent phage may be maintained by the existence of minority populations of sensitive bacteria that are more efficient in extracting resources than resistant bacteria. But if a virulent phage whose host range includes a dominant bacterial clone evolves or invades the community, then that clone will either evolve resistance or be replaced by another clone already resistant to that phage. Thus, resource-limited communities predominate, whereas communities in which the dominant bacterial clones are sensitive to co-occurring phage are transient aberrations.

5.1. Patterns of Abundance

An ideal data set for examining the role of phage in limiting bacteria in a natural community would include: (1) the host range of all phage determined for all bacteria; (2) all phage classified as temperate or virulent; (3) all bacteria insensitive to any phage classified as envelope resistance, restriction immunity, or the like; and (4) abundances of all bacteria and phage enumerated *in situ* over several sampling dates, with any changes in phage host range or bacterial sensitivity determined to be either an invasion of a new population or a genetic change in an existing population. I know of no study that contains anywhere near all of this information, but there are bits and pieces that provide some insights into the structure of certain phage–bacteria communities outside the laboratory.

Scarpino (1978) has reviewed studies in which densities of coliform bacteria and coliphage were estimated in sewage. Ratios of coliphage to coliforms are generally much less than 1. This is similar to most laboratory communities in which the dominant bacterial clone is resistant to co-occurring virulent phage; it is quite different from most laboratory communities in which the dominant bacterial clone is sensitive to co-occurring virulent phage (Table I). While this is not proof, it does suggest that the dominant coliform populations in sewage communities are not phage-limited, consistent with the prediction based on the coevolutionary asymmetry.

There is a striking difference in the relative abundance of temperate and virulent phage in sewage and feces. Whereas virulent forms predominate in sewage, temperate forms predominate in feces (Table III). There are two hypotheses that could account for the difference in phage composition between these environments. According to one, sewer and gut communities differ not in the dynamics *per se* of bacteria and phage populations, but rather in the rate at which these communities are invaded by new bacteria and phage populations. A gut is a relatively closed habitat, at least by comparison to a sewer. Invasion by virulent phage may drive sensitive bacteria extinct, thereby causing their own subsequent extinction. Invasion by temperate phage may result in the formation of lysogens, which are immune to reinfection, ensuring the persistence of both bacteria and phage. In sewers, there would be a continual supply of invading bacteria and phage, with "blooms" in virulent phage density commonplace, and extinctions obscured by the high rate of turnover. In the gut, by contrast, virulent phage blooms would be much less frequent, and extinctions more apparent. Sewage would thus contain a higher proportion of virulent phage than feces. While this is consistent with the hypothesis that "Lysogeny provides a solution to the problem of exhaustion of the host supply" (Echols, 1972), it assumes that virulent phage usually drive their sensitive bacterial hosts extinct. But we saw in Section 4.1 that virulent phage and bacteria usually coexist in chemostat and even serial culture. If extinctions are atypical in these simple, relatively homogeneous habitats, it seems difficult to support a hypothesis that relies on high rates of extinction in much more complex and heterogeneous habitats such as the gut.

Alternatively, the contrast in phage composition between sewage and feces could reflect more fundamental differences in phage infectivity. Perhaps the intestinal environment is simply less able to support lytic replication of phage. This might be related to the biochemistry of the

Table III. Comparison of the Relative Abundance of Virulent and Temperate Phage in Sewage and Feces[a]

	Number of isolates	Percent temperate
Feces		
Furuse *et al.* (1983)		
Healthy humans	150	90
Human patients	101	11
Dhillon *et al.* (1976)		
Humans	(17)[b]	(82)[b]
Nonhumans	(29)[b]	(45)[b]
E. S. Anderson (1957)		
Humans	6	83
Sewage		
Dhillon *et al.* (1970)	77	1
E. S. Anderson (1957)	6	0

[a]Number of isolates refers to the number of single-plaque isolates characterized as virulent or temperate, and percent temperate is the percent of these isolates found to be temperate. E. S. Anderson (1957) isolated phage on *Salmonella;* all others used *E. coli.*

[b]Data not specified by single-plaque isolates; values indicate percent of samples in which phage were detected that included some or all temperate phage.

medium, to the nutritional status of the bacterial hosts, or to the structure of the physical environment. Phage rely on diffusion by random Brownian motion to encounter bacteria, and diffusion is limited by high viscosity. Any colloidal matter that bound phage would further limit adsorption. Roper and Marshall (1974) have demonstrated that sensitive bacteria are protected from phage in the presence of organic sediment, because phage and/or bacteria are adsorbed to the sediment and prevented from direct contact with one another. Thus, it is possible that many phage simply cannot be supported by lytic replication in the gut because the rate at which they encounter suitable hosts is too low. Whatever the explanation, it is clear that sewers and guts are very different in the phage–bacteria interactions that they support, and that more study of the causes of these differences is required.

Orpin and Munn (1974) observed the invasion of a bacteriophage active against a large bacterium (designated EO2) that is characteristic of the rumen of sheep. The density of EO2 in the rumen of a sheep dropped from about 10^7 ml^{-1} to zero within 10 days of the phage invasion, whereas no such decline occurred in a sheep whose rumen was not invaded by this phage. About 1 month subsequent to the disappearance of EO2, it reappeared, although it did not attain the high density previously observed. The authors did not detect any significant replacement of the EO2 population by competitors. It may be significant, however, that the sheep used in this experiment had been "cleansed" of their normal rumen biota and then reinoculated with defined bacterial populations. In particular, a closely related bacterium, EO1, apparently resistant to the phage, was not present in the experimental animals.

Emslie-Smith (1961) identified a clone of *E. coli* that was dominant in the feces of a human subject for 8 months. A mixture of phage active against this clone was administered orally, resulting in the disappearance of this clone and its replacement by another clone, presumably resistant to the phage, that remained dominant for some time. There is no mention of whether any of the phage were able to replicate and establish self-sustaining populations in the gut.

Do there exist in nature resistant bacterial mutants for which there are no corresponding host-range phage mutants? Perhaps the best data on sensitivity and host-range comes from the work of Reanney and co-workers on *Bacillus* and soil phage isolated from the same local environments (Reanney, 1976; Tan and Reanney, 1976). Many of the phage types had broad host ranges, and most of the bacterial strains were sensitive to one or more co-occurring phage types. However, Tan and Reanney (1976) indicate that a large fraction of the bacteria belonged to one strain that was resistant to all of the phage types that were characterized from the same local environment. Moreover, free phage were difficult to isolate without enrichment, suggesting that many of the phage were temperate (or pseudolysogenic).

5.2. Phage Therapy Revisited

Whereas bacteriophage have been tremendously important in basic scientific research, their practical significance is relatively minor and often indirect. Phage may be useful, as agents for determining the clonal identity of bacterial pathogens (Milch, 1978) and as indicators of contamination (Scarpino, 1978). Phage may be harmful, as vectors for genes that confer antibiotic resistance or toxin production to pathogenic bacteria (Williams Smith, 1972; Freeman, 1951) and as contaminants of processes that rely on bacterial metabolism, such as cheese-making (Whitehead, 1953). But there was a time when phage were perceived as a "magic bullet" against bacterial disease.

Bacteriophage were discovered in 1915 by Twort and in 1917 by d'Herelle (Duckworth, 1976). Almost immediately, they were seized upon as means for controlling bacteria, stimulated in part by d'Herelle's (1922) claim that bacteriophage form the natural basis of immunity to infectious disease. According to d'Herelle (Stent, 1963), "Pathogenesis and pathology of dysentery are dominated by two opposing factors: the dysentery bacillus as pathogenic agent and the filtrable bacteriophage as agent of immunity." Within a few years, hundreds of papers appeared in medical journals from around the world concerning phage and their medical significance (Peitzman, 1969); phage were isolated that could infect the bacteria responsible for cholera, diphtheria, gonorrhea, plague, and other dreaded diseases (Stent, 1963). In 1925, Sinclair Lewis published a popular novel, *Arrowsmith,* in which a young doctor by that name undertakes the implementation of phage therapy. By the 1930s, at least three major pharmaceutical companies were marketing phage preparations in the United States, as was an international firm founded by d'Herelle (Peitzman, 1969). Despite this promising start, the outlook began to change dramatically. Perhaps most importantly, there was little convincing evidence that phage therapy produced any demonstrable benefit, as determined by reviews commissioned by the American Medical Association (Peitzman, 1969). [The use of phage in the control of bacteria that are agricultural pests also appears to have been largely unsuccessful (Vidaver, 1976).] At best, benefits were inconsistent, and scientific claims lacked proper controls. Peitzman (1969) concludes that "sulfonamides and antibiotics served as the final forces in the demise of bacteriophage therapy."

Although poor science and the advent of antibiotics certainly contributed to the historical failure of phage therapy, they do not provide a

biological explanation for this failure. In fact, phage would seem to have two advantages over antibiotics for the control of bacterial populations. First, phage are self-replicating entities, hence making their continual application unnecessary. Second, phage are evolving entities, hence counteracting the evolution of resistance by bacteria. However, a number of other factors could offset these advantages.

We have seen that temperate phage are much more common than virulent phage in feces (but not sewage), apparently because either the survival or the adsorption of free phage is inhibited *in vivo*. Although the factors responsible for this inhibition are not clear, they could have hindered certain therapeutic applications of phage. More generally, it is appropriate to recognize a fundamental difference between chemical and biological methods of control. Whereas both chemical and biological agents must adversely affect the target population, only biological agents must be adapted to the environment where they are to be released. It is not enough simply to find a "natural enemy" of the target organism. This point is well-illustrated by efforts to introduce parasites and predators of insect pests. DeBach (1971) reports that only about one-quarter of introduced parasites and predators have become established, and of these only about one-sixth (or 4% of the total) have exerted significant control over the target population. [Despite these low percentages, the benefit-to-cost ratios of biological control programs have been substantially higher than for chemical pesticides (DeBach, 1974).] We may similarly expect that only a small fraction of phage that can infect a bacterium *in vitro* would exert significant control *in vivo*.

We saw in Sections 2.2 and 4.2 that the coevolutionary potential of phage appears often to be less than that of their bacterial hosts; that is, there exist bacterial mutants resistant to phage for which there do not exist corresponding host-range phage mutants. This asymmetry could also have limited the efficacy of phage therapy. Furthermore, it is important to realize that most of the efforts at phage therapy were conducted *before* Lwoff (1953) had clearly established the phenomenon of lysogeny. Thus, it is quite possible that many of these efforts utilized temperate phage, and thereby generated lysogenic bacteria immune to reinfection.

In spite of the past history of failure, there has been some resurgence of interest in phage therapy in the last few years. This is due, at least in part, to the increasing prevalence of bacteria resistant to antibiotics, which has resulted primarily from the spread of plasmids (E. S. Anderson, 1968; Falkow, 1975). Bacteria responsible for nosocomial (hospital-acquired) infections are especially likely to be resistant to antibiotic therapy, and they plague patients whose immune systems are depressed; Shera (1970), for example, has explored the use of phage in combatting infections in burn patients.

Whether these newer (and more limited) applications can be successful remains to be seen. Success will almost certainly require the careful screening of possible phage agents, and not simply the isolation of any phage that happens to infect the target bacterium. (Advances in genetic engineering raise the possibility that appropriate phage might be constructed, rather than isolated *per se*.) In contrast to choosing an antibiotic, where a broad spectrum of sensitive bacteria is sought, the choice of an appropriate phage is likely to be specific to the target bacterium. One especially promising step in this direction has been taken by Williams Smith and Huggins (1982), who used phage successfully to treat experimental intramuscular and intracerebral infections in mice caused by a pathogenic strain of *E. coli*. They precluded the rise of resistant bacterial mutants by their very clever choice of phage. Pathogenicity of their target bacterium had been demonstrated previously to depend on the presence of the surface antigen K1 (Williams Smith and Huggins, 1980). Phage used to treat the infections were chosen because they adsorbed specifically to this antigen; bacterial mutants resistant to the phage lost the antigen, and hence their pathogenicity. Other phage that did not adsorb to this antigen were much less effective in treating these infections. In essence, Williams Smith and Huggins (1982) chose the phage such that there was a significant tradeoff between the resistance of the bacteria to the phage and the pathogenicity of the bacteria.

6. Summary

Bacteria and phage provide a powerful system for examining the dynamics of interacting populations, due in part to the strong conceptual and methodological framework provided by research in microbial genetics and molecular biology. The existence of phage-resistant bacterial mutants and extended host-range phage mutants is compelling evidence for their reciprocal adaptation, whereas the genetic and molecular characterizations of these mutants indicate significant constraints on their coevolution. In particular, there may exist phage-resistant bacteria for which there are not corresponding host-range phage mutants; and phage-resistant bacteria may have more limited metabolic capabilities than phage-sensitive bacteria.

The basic question that I have attempted to address is: How effective are phage infections in limiting the abundance of bacteria? I have focused on virulent phage because lytic infections are simpler and more adverse to bacteria than are lysogenic infections, which characterize temperate phage. However, the existence of temperate phage raises a number of other interesting questions that I have not addressed, concerning their own evolution and their role in bacterial evolution.

It is possible to modify the familiar Lotka–Volterra equations to pro-

vide a more realistic model of the interaction between bacteria and virulent phage. In particular, it is critical that the relation between bacteria and their own resources be incorporated. According to the model, virulent phage can coexist with sensitive bacteria in continuous culture, and may hold the density of sensitive bacteria well below that allowed by their resources. The model also predicts that phage-sensitive and phage-resistant bacteria can coexist in the presence of virulent phage, provided that the sensitive bacteria can outcompete the resistant bacteria in the absence of phage.

Bacteria and virulent phage populations generally coexist in laboratory communities. Phage-limited bacteria typically occur at densities several orders of magnitude below that set by their resources; phage outnumber sensitive bacteria often by two orders of magnitude. Equilibrium population densities can usually be predicted with reasonable accuracy by independently estimating the parameters of the model, but the dynamics appears to be generally more stable than anticipated from theory. The factors responsible for discrepancies between theory and experiment have not yet been determined, although nongenetic variation in the vulnerability of individual bacteria to phage infection could be important.

Evolution in coexisting bacteria and virulent phage populations can be very rapid and may have profound effects; for example, the appearance of a bacterial genotype that is resistant to all phage genotypes can increase the total bacterial density by several orders of magnitude. The study of coevolving laboratory populations corroborates the existence of constraints on phage and bacteria, and demonstrates that these constraints are important in structuring communities. Phage-resistant bacterial mutants usually appear, including some for which no corresponding host-range phage mutants appear. These resistant bacteria are often at a pronounced competitive disadvantage relative to sensitive bacteria, permitting their coexistence and the maintenance of virulent phage. The resulting community can be described as resource-limited, but contains a minority population of phage-limited sensitive bacteria. Total bacteria typically outnumber phage by an order of magnitude or more in such communities.

Determining whether these constraints are important in structuring natural communities is much more difficult. If the significant features of phage–bacteria interactions in nature depend on particular characteristics of their environments (intestine, sewer, soil, and so on), then broad generalizations may be impossible. For example, virulent phage are less common than temperate phage in feces, whereas in sewage the opposite is true. This suggests that the survival of free phage or their adsorption to suitable bacteria is inhibited in the gut.

More generally, it does appear that many bacteria are resistant or otherwise insensitive to co-occurring phage in nature. Lytic phage infections do not obviously limit most bacterial communities to densities well below that set by their resources, although component populations may be limited. If further research supports this interpretation, then the evolutionary constraints documented in the laboratory may also be significant in many natural communities. Much more research is necessary, however, especially concerning: (1) details of the dynamics and trophic relationships of co-occurring phage and bacteria in nature; (2) effects of physically structured habitats on the interactions of bacteria and phage; and (3) consequences of recombination for the coevolution of bacteria and phage in genetically diverse communities.

Phage were advanced as agents for the control of bacteria soon after their discovery, but "phage therapy" has generally been regarded as a failure. Historical factors, including especially the advent of antibiotics, contributed to this failure. However, the biological factors responsible for the failure are not clear; their self-replicating and coevolving nature would seem to give phage certain advantages over antibiotics. The increasingly widespread resistance of bacteria to antibiotics has prompted renewed interest in the use of phage for their control.

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