CHAPTER 2

Bacteriophages: models for exploring basic principles of ecology

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2.1 INTRODUCTION

A virus depends intimately upon its host in order to reproduce, which makes the host organism a crucial part of the virus's environment. This basic facet of viral existence means that ecology, the scientific field focusing on how organisms interact with each other and their environment, is particularly relevant to the study of viruses. In this chapter we explore some of the ways in which the principles of ecology apply to viruses that infect bacteria – the bacteriophages (or "phages" for short). In turn, we also discuss how the study of phages and their bacterial hosts has contributed to different subfields of ecology.

Due to their ease of manipulation, large population sizes, short generation times, and wealth of physiological and genetic characterization, laboratory communities of microbial organisms have been popular experimental tools for testing ecological theory (Drake *et al.*, 1996; Jessup *et al.*, 2004). Building upon this foundation of knowledge, the ecological experimentalist can explore whether mechanistic understanding at the organismal level informs an understanding of patterns at the community level (Bohannan and Lenski, 2000a). Further, the initial composition of microbial consortia can be controlled, and thus researchers are able to probe the effects of different community structures on ecological phenomena, such as stability, diversity, and resilience to invasion.

In particular, microcosms of bacteria and phages have been used by many researchers to study the ecology of victim–exploiter interactions (Chao

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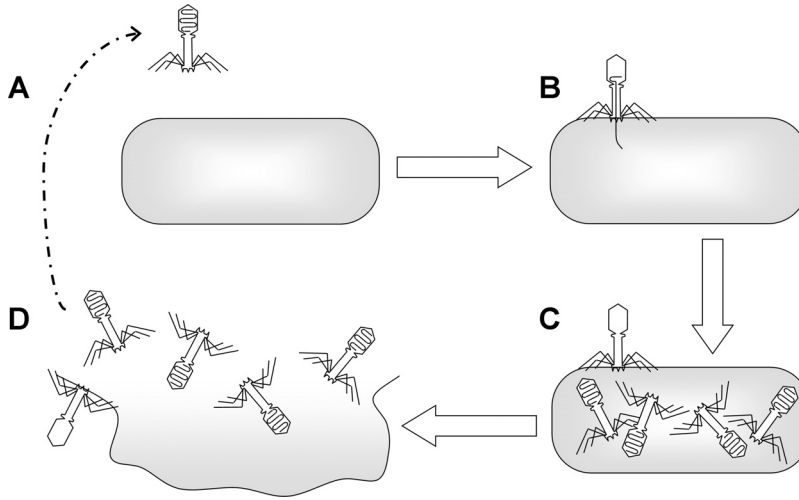

Figure 2.1 The life cycle of a typical lytic bacteriophage. (A) A single infective phage particle is shown with its bacterial host. (B) The phage binds to receptors on the surface of its host (adsorption) and injects its genome into the bacterial cytoplasm. (C) The bacterial cell becomes a phage factory, where the phage genome is copied, phage structural proteins are synthesized, and the genome is packaged. (D) At the end of the latent period (which starts with phage genome injection), the host cell lyses, releasing the progeny phages. A progeny phage particle can then infect a new host, starting the cycle anew (dot-dashed arrow).

et al., 1977; Levin *et al.*, 1977; Lenski and Levin, 1985; Schrag and Mittler, 1996; Bohannan and Lenski, 1997, 1999, 2000b). These relationships are ubiquitous within ecosystems (e.g., prey and their predators, plants and their herbivores, hosts and their pathogens, etc.). In this chapter, we will focus on the obligately lytic (*sensu* Chapter 1) bacteriophages of *Escherichia coli*. The archetypical life cycle is illustrated in Fig. 2.1. Here, we discuss how ecological theory and concepts apply to these phages and how viruses have inspired new conceptual developments within ecology.

2.2 MATHEMATICAL ECOLOGY

Since ecologists deal with complex systems of interacting species, mathematical frameworks have proved extremely useful for both predictive purposes and conceptual understanding. Studies of victim–exploiter interactions have a particularly distinguished history within mathematical ecology, from the continuous-time theory of predators and their prey (Lotka, 1925; Volterra, 1926) to the discrete-time theory of parasites and their hosts (Nicholson and

Bailey, 1935). Such mathematical models have highlighted some basic principles of victim–exploiter interactions, including the fundamental occurrence of oscillations and the factors contributing to community stability.

2.2.1 Lotka–Volterra modeling

One of the simplest mathematical models for understanding the dynamics of predators and their prey was formulated independently by Lotka (1925) and Volterra (1926). This model assumes that prey increase exponentially without predators present and that predators decrease exponentially without prey present. The model also assumes that the growth of the predator population is proportional to the food intake through predation, which depends on the likelihood of predator–prey encounters. If we assume mass action (the rate of interaction between predators and prey being proportional to their product), then the dynamics for predator density (P) and prey density (N) are:

$$\frac{dN}{dt} = \Psi \cdot N - \alpha \cdot N \cdot P \tag{2.1}$$

$$\frac{dP}{dt} = \beta \cdot \alpha \cdot N \cdot P - \delta \cdot P \tag{2.2}$$

with $\boldsymbol{\Psi}$ the growth rate of the prey, α the attack rate of predators, β the predator's efficiency in converting its food to offspring, and δ the death rate of the predator.

There is a single non-trivial equilibrium for this system:

$$(\hat{N}, \hat{P}) = (\delta/(\beta \cdot \alpha), \Psi/\alpha) \tag{2.3}$$

which is neutrally stable (i.e., small perturbations from the equilibrium are neither amplified nor damped). Interestingly, the equilibrial density of the prey depends only on the parameters of the predator and *not* on the growth rate of the prey itself. The full dynamics of this system consist of neutrally stable oscillations, the amplitude of which are determined by the initial densities of predators and prey (May, 1974; Edelstein-Keshet, 1988; Fig. 2.2A). This model has been criticized for being (1) biologically oversimplistic and (2) incapable of explaining sustained oscillations (with neutrally stable cycles, a perturbation of either predator or prey to low density gives rise to cycles regularly visiting low densities, which increases the chance of extinction; see Fig. 2.2A). However, as this model sits on the border between stability and instability, it is a conceptually useful baseline from which to explore stabilizing and destabilizing factors in predator–prey relationships (Edelstein-Keshet, 1988). For instance, if the prey's per capita growth rate decreases as

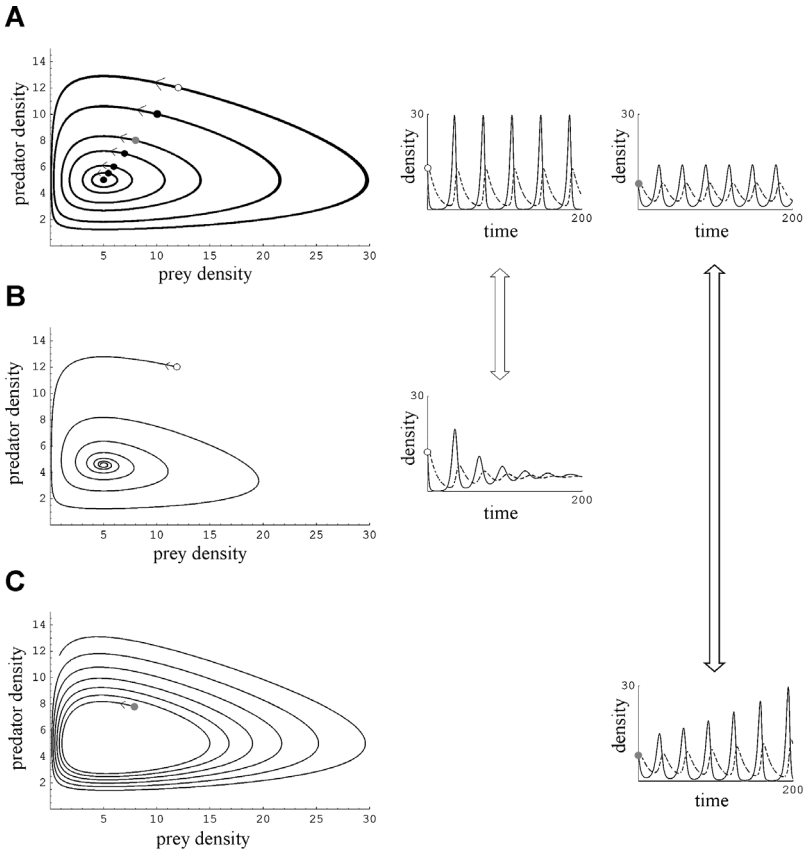

Figure 2.2 Dynamics of a predator–prey community. (A) We use the standard δ Lotka–Volterra model: $dN/dt = \Psi \cdot N - \alpha \cdot N \cdot P$, $dP/dt = \beta \cdot \alpha \cdot N \cdot P - \delta \cdot P$, with $\Psi = 0.5$, $\alpha = 0.1$, $\beta = 0.2$, and $\delta = 0.1$. In the main graph, we show stable limit cycles in a phase plane, where initial densities of the predator and prey determine the amplitude of the cycles (thick lines). The filled circles give the various starting conditions: $N_0 = P_0 = 5$ (the neutral equilibrium), $N_0 = P_0 = 5.5$, $N_0 = P_0 = 6$, $N_0 = P_0 = 7$, $N_0 = P_0 = 8$ (marked with the gray circle), $N_0 = P_0 = 10$, and $N_0 = P_0 = 12$ (marked with the white circle). The small graphs to the right of the phase plane give the time trajectories for the starting conditions $N_0 = P_0 = 8$ (gray circle) and $N_0 = P_0 = 12$ (white circle), where the bacterial density is a solid line and the phage density is a dashed line. (B) We change the model slightly to add negative density dependence for the prey: $dN/dt = \Psi \cdot N(1 - N/K) - \alpha \cdot N \cdot P$, $dP/dt = \beta \cdot \alpha \cdot N \cdot P - \delta \cdot P$, with $\Psi = 0.5$, $\alpha = 0.1$, $\beta = 0.2$, $\delta = 0.1$, and the carrying capacity $K = 60$. Starting with $N_0 = P_0 = 12$ (the white circle), we see that the community exhibits damped oscillations (see also the small graph to the right). Compared to the standard model, negative density dependence stabilizes the predator–prey dynamics (the double-edged arrow points to the graph of the

its density increases (negative density-dependent regulation) then the system is stabilized; on the other hand, a time lag in the processing of the prey can destabilize the system (May, 1972, 1974; Holling, 1973; Fig. 2.2B and C; Chapter 15).

2.2.2 Modeling predator–prey dynamics using phages

Communities of bacteriophages and bacteria serve as ideal test systems of predator–prey theory, and several mathematical models have been tailored to explore the dynamics of these microbial communities (Campbell, 1961; Chao *et al.*, 1977; Levin *et al.*, 1977; Lenski and Levin, 1985; Schrag and Mittler, 1996; Bohannan and Lenski, 1997, 1999, 2000b; Chapter 15). Many of these models make the assumption that the microbes inhabit a "chemostat-like" environment – one which is spatially homogeneous, constant in abiotic variables and continuously supplied with nutrients. In the chemostat, medium containing resources flows into a vessel at a constant rate and depleted medium flows out at the same rate, such that a stable vessel volume is maintained (Chapters 9 and 15). In Section 2.8 we give an example of a continuous-time chemostat model.

As was the case for the simple Lotka–Volterra model, chemostat models predict that equilibrium bacterial density is a function of phage effectiveness only (Levin *et al.*, 1977; Lenski and Levin, 1985; Equation 2.3). Also, these models incorporate explicitly some of the stabilizing and destabilizing factors mentioned above. For instance, bacteria compete for limiting resources and thus there is negative density-dependent regulation in the chemostat. Indeed, the limiting resource concentration in the vessel is often modeled as a dynamic variable, where its rate of change is negatively related to bacterial density (Section 2.8). While such density dependence is a stabilizing force, time lags inherent to this system destabilize dynamics. One fundamental

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Figure 2.2 (*continued*)
standard model and the graph of the negative-density-dependence model for the same starting conditions). (C) We now add a time delay: $dN/dt = \Psi \cdot N - \alpha \cdot N \cdot P$, $dP/dt = e^{-\tau \cdot \delta} \beta \cdot \alpha \cdot N' \cdot P' - \delta \cdot P$, with $\Psi = 0.5$, $\alpha = 0.1$, $\beta = 0.2$, $\delta = 0.1$; the time delay (for the predator to convert captured prey items into predator progeny) is $\tau = 0.17$, and the primed variables are evaluated τ time units in the past (i.e., $N' = N(t - \tau)$ and $P' = P(t - \tau)$). Starting with $N_0 = P_0 = 8$ (the gray circle), we see that the community exhibits expanding oscillations (see also the small graph to the right). Compared to the standard Lotka–Volterra model, a time delay destabilizes the predator–prey dynamics (the double-edged arrow points to the graph of the standard model and the graph of the time-lag model for the same starting conditions).

time lag involves the latent period of the phage – the interval between phage "capture" of a bacterial prey (Fig. 2.1B) and the transfer of that capture into phage progeny (Fig. 2.1D; Chapter 15). As a consequence of this lag, phage–bacteria dynamics are generally written as time-delay differential equations (Section 2.8).

If coexistence is predicted within these models, then generally both predator and prey either approach fixed densities (stable fixed point) or both parties cycle in steady fashion (stable limit cycle). However, sometimes these cycles reach densities so low that extinction, due to finite population size, is predicted. In actual chemostats, phages and bacteria tend to coexist (sometimes for long intervals), approaching semi-fixed densities or undergoing regular cycles (Horne, 1970; Paynter and Bungay, 1971; Chao *et al.*, 1977; Levin *et al.*, 1977; Lenski and Levin, 1985; Schrag and Mittler, 1996; Bohannan and Lenski, 1997, 1999, 2000b; Fig. 2.4). Such coexistence can occur even when theory predicts otherwise (Levin *et al.*, 1977; Schrag and Mittler, 1996; Bohannan and Lenski, 1997, 2000b). In such cases, the theoretical framework is likely missing key components, such as spatial, numerical, or physiological refugia from phage attack (Lenski and Levin, 1985; Lenski, 1988; Schrag and Mittler, 1996; Bohannan and Lenski, 2000a). For example, hidden layers of surface growth on the walls of the chemostat may serve as a spatial refuge for phage-sensitive bacteria (Schrag and Mittler, 1996).

2.2.3 More complex phage-based models

The models employed in Section 2.8 (Appendix) consider only a single type of predatory phage and a single vulnerable strain of bacteria. However, there is frequently evolution of new members within laboratory microbial communities, such as fully or partially phage-resistant bacterial mutants and host-range phage mutants. In fact, in nearly all the chemostat studies listed above there was evidence of phage-resistant bacterial mutants arising during the experiment. To probe this added complexity, some researchers have increased the number of dynamic players in their models (Levin *et al.*, 1977; Bohannan and Lenski, 1999, 2000b). For example, Levin *et al.* (1977) constructed a model with an arbitrary number of predatory phage species, prey bacterial species, and resources. They found that, at equilibrium, the number of phage species could not exceed the number of bacterial species; and the number of bacterial species could not exceed the sum of the number of resources and the number of phage species. This "exclusion principle" applies only to equilibrium conditions, and violations of the principle have

been described in communities exhibiting periodic and chaotic dynamics (Armstrong and McGehee, 1980; Huisman and Weissing, 1999). Systems with multiple predators and multiple prey are a topic of interest in community ecology, a subject to which we now turn.

2.3 COMMUNITY ECOLOGY

Understanding how interactions among species affect the structure and spatio-temporal dynamics of biological communities is a core objective within community ecology. Many natural communities are a spaghetti-like tangle of interactions. One approach to unraveling this complexity involves the study of stripped-down communities comprising a smaller number of interacting species, so-called "community modules" (Holt *et al.*, 1994; Holt, 1995; Bohannan and Lenski, 2000a). In communities of predators and their prey, the examination of such modules has led to a deeper understanding of the circumstances in which different prey species negatively impact one another through a shared predator, as well as of the conditions under which a predator plays a "keystone" role in maintaining diversity (Holt, 1977, 1995; Holt *et al.*, 1994; Leibold, 1996).

2.3.1 Microbial modules

Microbial microcosms of bacteria and their viruses are well suited for the construction of module communities (Drake *et al.*, 1996; Bohannan and Lenski, 2000a; Jessup *et al.*, 2004). In laboratory settings, the experimenter can easily control the structure of the community by initiating the microcosm with a select set of interacting species. The experimenter can then track the population dynamics and monitor how the community structure changes over time. Such changes can occur when (1) invading species are intentionally added to an established community, (2) new community members evolve *de novo* within the microcosm, and (3) when community players become extinct.

Researchers using bacterium–phage modules have explored a number of topics of interest within community ecology. For instance, the conditions favoring bottom-up (resource-driven) versus top-down (predator-driven) control of prey populations have been investigated with phage and bacteria (Chao *et al.*, 1977; Bohannan and Lenski, 1997, 1999, 2000b). The impact of invading species on community structure and dynamics has been probed using these microbial modules (Levin *et al.*, 1977; Lenski and Levin, 1985;

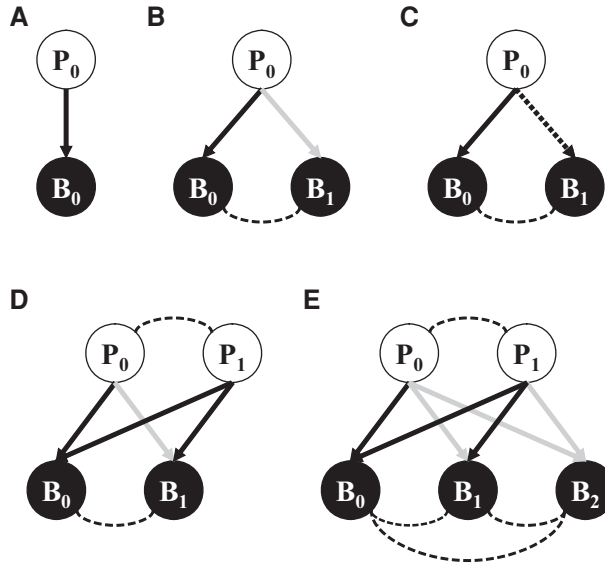

Figure 2.3 Different community modules. In each module, the phage predators are shown as empty circles and the bacterial prey are shown as filled circles. If a phage strain can infect a particular bacterial strain, then a black arrow points from the phage to the host (where a dotted arrow indicates a host *partially* resistant to phage entry). If the phage cannot infect a particular strain of bacterium, then a gray arrow points from the phage to the bacterial strain. Lastly, dashed lines connect strains that are "mutant neighbors" of one another (that is, strains that can mutate to become the attached strain). Shown are (A) the simple single phage strain and single sensitive bacterial strain, (B) a single phage strain, a sensitive bacterial strain, and a fully phage-resistant strain, (C) a single phage strain, a sensitive bacterial strain and a partially resistant strain, (D) two phage strains (an ancestor and a host-range mutant) and two bacterial strains: the phage ancestor can only attack one of the two bacterial strains, whereas the host-range mutant can attack both, and (E) two phage strains and three bacterial strains: exactly the situation in part D, but with an extra bacterial strain that is fully resistant to both phage strains.

Bohannan and Lenski, 1999). The competitive effects that take place between prey species through a common predator have also been explored (Levin *et al.*, 1977; Bohannan and Lenski, 2000b). Several researchers have reported the importance of phenotypic trade-offs in determining community structure (Chao *et al.*, 1977; Levin *et al.*, 1977; Lenski and Levin, 1985; Bohannan and Lenski, 1999). Finally, the effect of resource enrichment on stability and diversity has been studied within these microbial communities (Bohannan and Lenski, 1997, 1999, 2000b). Below we discuss these findings, organizing the experimental communities by their structural complexity (Fig. 2.3).

2.3.2 Communities with phages and sensitive bacteria

Most experimental microcosms are initiated with a single strain of bacteria and a single strain of phage (Fig. 2.3A). For instance, chemostats have been inoculated with *E. coli* and one of the following phage types: λ*vir* (Schrag and Mittler, 1996), T1X (Schrag and Mittler, 1996), T2 (Paynter and Bungay, 1971; Levin *et al.*, 1977; Lenski and Levin, 1985; Bohannan and Lenski, 2000b), T3 (Horne, 1970), T4 (Horne, 1970; Lenski and Levin, 1985; Bohannan and Lenski, 1997, 1999), T5 (Lenski and Levin, 1985), or T7 (Chao *et al.*, 1977; Lenski and Levin, 1985; Forde *et al.*, 2004). In most cases, a phage-resistant strain of bacteria evolved and increased in frequency (see next two sections); thus, a *de novo* increase in complexity occurred. However, in a few cases, some hypotheses about two-member community dynamics could be tested before invasion took place. For instance, Bohannan and Lenski (1997, 1999) found that resource enrichment led to a significant increase in T4 phage density and only a small increase in bacterial density. Furthermore, these authors observed that higher resource concentration destabilizes predator–prey dynamics, an example of the so-called "paradox of enrichment" (Rosenzweig, 1971; Section 2.8; Fig. 2.4A and B; Chapter 10).

2.3.3 Communities with fully resistant bacteria

As mentioned above, even in communities started with only a single sensitive bacterial strain and a single strain of phage, a phage-resistant bacterial strain often evolved and invaded. In some cases, a resistant strain was added to the microcosm and this initially rare species similarly increased in density (Levin *et al.*, 1977; Bohannan and Lenski, 1999). In these cases, the community module had three players, two consumer species (the bacteria) and one predator (the phage) that made a living on one of the consumers (Fig. 2.3B). In the majority of cases, this community maintained all three members (Paynter and Bungay, 1971; Levin *et al.*, 1977; Lenski and Levin, 1985; Schrag and Mittler, 1996; Bohannan and Lenski, 1999).

2.3.3.1 Trade-offs are stabilizing

What allows sustained coexistence? The invasion of the resistant strain occurs because it is invulnerable to the phage. However, why does the resistant bacterial strain not displace the sensitive strain? In most cases, this is due to a trade-off between competitive ability and resistance to phage infection (Bohannan *et al.*, 2002). A competitive cost to phage resistance has been shown for *E. coli* resistant to phage T2 (Levin *et al.*, 1977; Lenski, 1984; Lenski

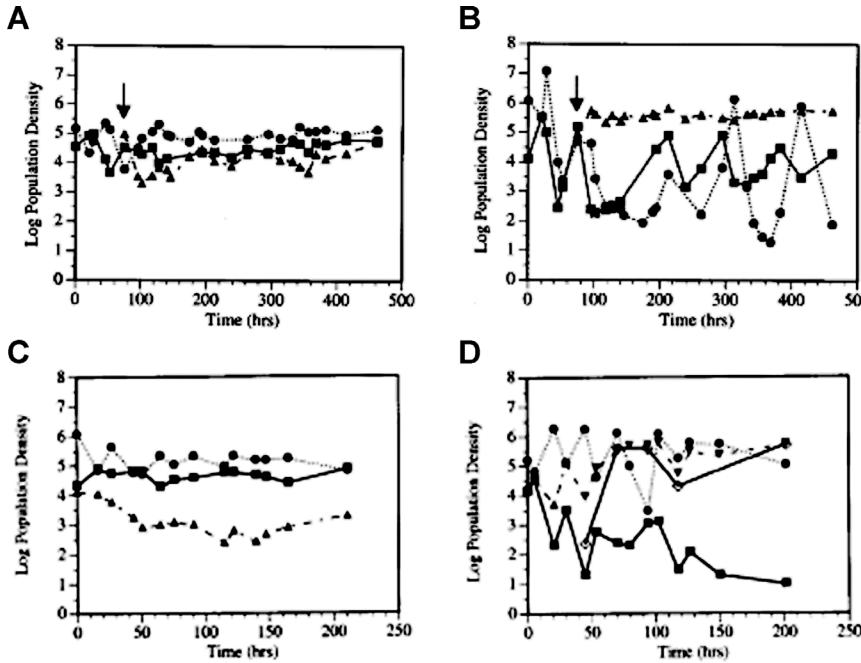

Figure 2.4 Chemostat community dynamics. (A) The dynamics of phage-sensitive *E. coli* B (squares), phage-resistant *E. coli* B (triangles), and phage T4 (circles) in glucose-limited chemostats with a reservoir concentration of 0.1 mg mL^{-1} (low productivity). The arrow indicates when phage-resistant bacteria were added to the chemostat. (B) The dynamics of the sensitive *E. coli*, resistant *E. coli*, and phage T4 in chemostats with a reservoir glucose concentration of 0.5 mg mL^{-1} (high productivity). (C) The dynamics of a sensitive *E. coli* B (squares), partially resistant *E. coli* B (triangles), and phage T2 (circles) in a chemostat with a reservoir glucose concentration of 0.1 mg mL^{-1}. (D) The dynamics of the sensitive *E. coli*, partially resistant *E. coli*, phage T2, and fully resistant *E. coli* (diamonds) in chemostats with a reservoir glucose concentration of 0.5 mg mL^{-1}. (Reprinted with permission from Bohannan and Lenski, 1999 [*Am. Nat.* **153**: 73–82], and Bohannan and Lenski, 2000b [*Am. Nat.* **156**: 329–340]. © 1999 and 2000, University of Chicago.)

and Levin, 1985), phage T4 (Lenski and Levin, 1985; Lenski, 1988; Bohannan and Lenski, 1999), and phage T7 (Chao *et al.*, 1977; Lenski and Levin, 1985). This cost has been shown to be a function of the particular mutation conferring resistance, the genetic background, and the abiotic environment (Lenski, 1988; Bohannan and Lenski, 2000a; Bohannan *et al.*, 2002). Trade-offs between competitive ability and protection from predation are found in other organisms as well (Grover, 1995; Kraaijeveld and Godfray, 1997; Gwynn *et al.*, 2005).

Such trade-offs are predicted by chemostat models to allow coexistence in these three-member modules (Levin *et al.*, 1977). The sensitive bacteria persist due to a higher growth rate than the resistant prey when the latter approach their resource-limited equilibrium, while the resistant bacteria persist due to phage control of the sensitive population. When a trade-off between phage resistance and competitive ability does not exist, the community can lose members. For example, resistance to phage T5 by *E. coli* B comes without a competitive cost in glucose-limited laboratory culture; indeed, this is why T5 resistance is such a popular genetic marker in *E. coli* (Bohannan and Lenski, 2000a). Lenski and Levin (1985) showed that the advent of T5-resistant bacteria in chemostats initiated with phage T5 and T5-sensitive bacteria led to the eventual extinction of both phage and sensitive bacteria.

2.3.3.2 Top-down versus bottom-up control

The presence of a fully resistant bacterial strain is also predicted to shift regulation of prey from top-down (predator control) to bottom-up (resource control). Specifically, resource enrichment is predicted to lead to an increase in the predator density when only a single susceptible prey species is present (Fig. 2.3A), whereas enrichment is predicted to lead only to an increase in the invulnerable prey when both susceptible and invulnerable prey species live with a predator (Leibold, 1989). Bohannan and Lenski (1999) showed that these predictions were qualitatively accurate for a chemostat community of phage T4 and a heterogeneous bacterial population. Furthermore, these authors demonstrated that a version of the "paradox of enrichment" occurred in a subset of their community. Specifically, the phage and sensitive bacteria dynamics were destabilized by increased resource input into the chemostat vessel (whereas the dynamics of the phage-resistant bacterial strain were stabilized with enrichment).

2.3.4 Communities with partially resistant bacteria

Phage T2 can bind to two different receptors on the surface of its bacterial host (Lenski, 1984). This predatory flexibility enables bacteria to evolve partial resistance to T2, in which one of the two receptors has been lost or altered. In communities containing phages, sensitive bacteria, and partially resistant bacteria (Fig. 2.3C), the prey species interact through two indirect routes. First, the bacterial strains compete for resources, a phenomenon termed "exploitative competition" (Holt *et al.*, 1994). Second, each bacterial strain feeds a predator that attacks the other strain, a phenomenon termed "apparent

competition" (Holt, 1977; Holt *et al.*, 1994). Levin *et al.* (1977) describe a counterintuitive situation in which the *decrease* in resistance of a partially resistant strain leads to a slight increase in its density and a dramatic decrease in the density of the completely sensitive strain in the presence of a common phage predator. Here the maxim "the enemy of my enemy is my friend" has particular salience. The partially resistant strain gains the upper hand against its superior competitor (the completely sensitive strain) by acting as a phage "carrier." In this scenario, the inferior competitor "delivers" the predator to the more susceptible strain (Levin *et al.*, 1977).

Bohannan and Lenski (2000b) investigated the effect of resource enrichment (i.e., an increase in the input concentration of growth-limiting resource) on the dynamics of these three-member modules. Under low resource input, competition between prey species is expected to influence community patterns; whereas under high resource input, predation is expected to exert a stronger shaping force (Holt *et al.*, 1994; Leibold, 1996). In the absence of phages, bacterial strains partially resistant to phage T2 incur a competitive disadvantage relative to fully sensitive strains (Bohannan and Lenski, 2000b). Bohannan and Lenski predicted that an increase in resource input would shift a community from one where the partially resistant strain was excluded by the sensitive strain to one where the sensitive strain was excluded by the partially resistant strain, with a narrow region of coexistence at intermediate resource input. While they did not observe exclusions, their data did suggest that the sensitive strain (the superior competitor) fared better at low resource input and that the partially resistant strain (the less susceptible) fared better at high resource input (Fig. 2.4C and D).

2.3.5 Communities with phage host-range variants

Even after the evolution of fully resistant bacteria, some bacteriophages can evolve a counter-response, in which mutants can infect the previously resistant bacterial strain. Such host-range mutants have been reported in phage T2 and phage T7 (Chao *et al.*, 1977; Lenski and Levin, 1985). In chemostats inoculated with sensitive *E. coli* B (B_0) and phage T7 ($T7_0$), Chao *et al.* (1977) witnessed the evolution of T7-resistant bacteria (B_1) and then the evolution of phage that could infect B_1 ($T7_1$). In such a four-member community (Fig. 2.3D), prey regulation seemed to be largely top-down, with low densities of bacteria maintained. However, in some of their chemostats, another bacterial strain (B_2) evolved, which was resistant to both $T7_0$ and $T7_1$. In this five-member community (Fig. 2.3E), prey regulation seemed to be primarily bottom-up, with high bacterial densities found.

These four- and five-member communities persist experimentally (Chao *et al.*, 1977), and the importance of phenotypic trade-offs resurfaces (Section 2.3.3.1). Bacterial strains with resistance (B_1 and B_2) are competitively inferior to the fully sensitive strain (B_0). The phage host-range mutant ($T7_1$) is competitively inferior to its ancestor ($T7_0$) in competition for sensitive hosts (B_0). Using a combination of adaptive dynamics and stochastic simulations, Weitz *et al.* (2005) demonstrated that a large number of host-range phage mutants and partially resistant bacterial strains can theoretically coexist. This occurs if the growth costs accompanying partial host resistance to ancestral phages are small relative to the host-range trade-offs in phages (where higher adsorption on one host can result in lower adsorption on a different host). Indeed, the existence of trade-offs may be a general theme in the maintenance of diversity (Tilman, 2000; Bohannan *et al.*, 2002).

The coevolutionary arms races exhibited by phages such as T7 appear lop-sided, with the bacterial host having the last word (Bohannan and Lenski, 2000a). This is because bacteria have the option to alter or lose a phage receptor. While a phage may be able to cope with receptor alterations, the complete loss of a sole binding site is a difficult challenge to answer evolutionarily (although not impossible; see Morona and Henning, 1984). At this point, we should mention that there are other meaningful evolutionary changes that take place in phages besides host-range shifts. Some mutations will alter how the virus uses its bacterial host during its infection; that is, its "foraging strategy," which is the subject of the next section.

2.4 BEHAVIORAL ECOLOGY

All organisms must secure resources in order to develop, survive, and reproduce. Consequently, how organisms find and use resources are critical components of any fitness measure. At least in part, the success of the foraging strategy employed will depend on the organism's environment. In particular, the quality, quantity, distribution, permanence, and heterogeneity of critical resources will influence the optimal foraging strategy; and there has been much theoretical progress towards understanding these relationships (MacArthur and Pianka, 1966; Charnov, 1976; Stephens and Krebs, 1986).

2.4.1 Optimal foraging theory

As a concrete example of foraging-strategy optimization, imagine a hummingbird foraging for nectar in a landscape in which flowers are patchily

distributed (Fig. 2.5). Assume that travel from patch to patch takes a certain amount of time, d (dispersal time). Once inside a patch, our hummingbird starts to feed. However, due to a limited amount of nectar, the energetic gain over time shows diminishing returns (the concave function in Fig. 2.5A). How long should the hummingbird stay inside one patch before leaving for the next one? That is, what is its optimal "patch residence time" (or t)? This residence time determines the energetic gain from the patch; specified by the function $g(t)$ in Fig. 2.5A (note, we assume $g(0) = 0$, $g'(t) > 0$, and $g''(t) < 0$). Using the rate of energetic gain (r) as a proxy for fitness, we would like to determine the value of t (call it t^*) that maximizes the quantity $r(t) = g(t)/(d + t)$.

It turns out that $r(t)$ can be visualized graphically: simply draw a line from the filled point on the x-axis a distance d behind the origin to the point $(t, g(t))$; the slope of the resulting line gives $r(t)$. If the residence time is very short or very long (e.g., t_{short} and t_{long}, respectively, in Fig. 2.5A), then the slope is shallow and $r(t)$ is small. The optimal residence time is the value t^* such that the aforementioned line is tangent to the gain function (Fig. 2.5A). This is the marginal value theorem (Charnov, 1976), which states that the optimal residence in a patch is given by the time at which the instantaneous rate of energetic gain (the slope of the tangent to $g(t)$) is equivalent to the long-term rate of energetic gain (the slope of the line connecting the filled point on the x-axis to the point $(t, g(t))$.

We can now ask what would happen to our hummingbird's optimal residence time if its world changes. What happens if the dispersal time between patches becomes longer (compare Fig. 2.5C with Fig. 2.5B) or patch quality increases (compare Fig. 2.5D with Fig. 2.5B)? As shown in Fig. 2.5E and F, a longer dispersal time leads to an increase in optimal residence time, whereas an increase in patch quality leads to a decrease in optimal residence time.

2.4.2 Optimal "phoraging" theory

How might the marginal value theorem apply to bacteriophages? In a very real sense, bacteriophages are foragers of "bacterial resource patches." The time from host lysis to subsequent infection is a dispersal period, D (i.e., the time from Fig. 2.1D to Fig. 2.1B). The dispersal period is inversely proportional to host cell density (Fig. 2.6) and the rate of phage adsorption. The "residence time" of the virus is its latent period, L (i.e., the time from the state shown in Fig. 2.1B to the state shown in Fig. 2.1D). The latent period is generally broken into two periods: (1) the eclipse period, E, which spans

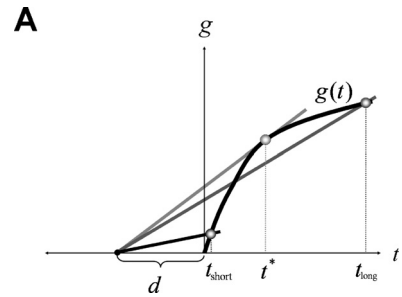

Figure 2.5 Optimal foraging theory. (A) Assume that the time to travel from patch to patch is d, which is why we place a point on the abscissa a distance d to the left of the origin. The thick curve, $g(t)$, gives the energetic gain (on the ordinate) as a function of time spent in the patch (on the positive abscissa). Now, it is simple to graphically describe the long-term average energetic gain, $r(t)$. For a residence time of t_0, simply connect the two points $(t_0, g(t_0))$ and $(-d, 0)$: the slope of this line is $r(t_0) = g(t_0)/(t_0 + d)$. With a concave increasing gain function, we see that short residence times (e.g., t_{short}) or long residence times (e.g., t_{long}) give suboptimal slopes (the shallow black and blue lines, respectively). The maximal slope is obtained for the residence time ($t = t^*$) in which the connecting line is also tangent to the gain curve, in this case the steeper red line. (B) In environment 1, the hummingbird encounters relatively low-productivity patches (with gain function $g_1(t)$) with a relatively short dispersal time between patches (d_1). (C) In environment 2, the hummingbird encounters low-productivity patches (with gain function $g_2(t) = g_1(t)$), but with a longer dispersal time between patches ($d_2 > d_1$). (D) In environment 3, the hummingbird encounters more productive patches ($g_3(t) > g_1(t)$ for all $t > 0$), but with a relatively short dispersal time ($d_3 = d_1$). (E) When we compare optimal residence time in environment 1 (the point of tangency of the purple line, ${t_1}^*$) to the optimal residence time in environment 2 (the point of tangency of the green line, ${t_2}^*$), we see that increasing the dispersal time tends to increase the optimal residence time. (F) When we compare optimal residence time in environment 1 (the point of tangency of the purple line, ${t_1}^*$) to the optimal residence time in environment 3 (the point of tangency of the orange line, ${t_3}^*$), we see that increasing the patch productivity tends to decrease the optimal residence time. *See color plate section.*

the time from initial infection (Fig. 2.1B) to the maturation of infective progeny phage within the host (prior to Fig. 2.1C) and (2) the adult period, A, which lasts from initial progeny maturation to host lysis (Fig. 2.1D). During the adult phase, progeny are produced at a rate, R (see Chapters 3 and 15 for additional discussion of these various phage growth-parameter concepts). Here, we will assume that the number of progeny released at host lysis (the burst size, B) is a linear function of L, namely

$$B(L) = R \cdot (L - E) \tag{2.4}$$

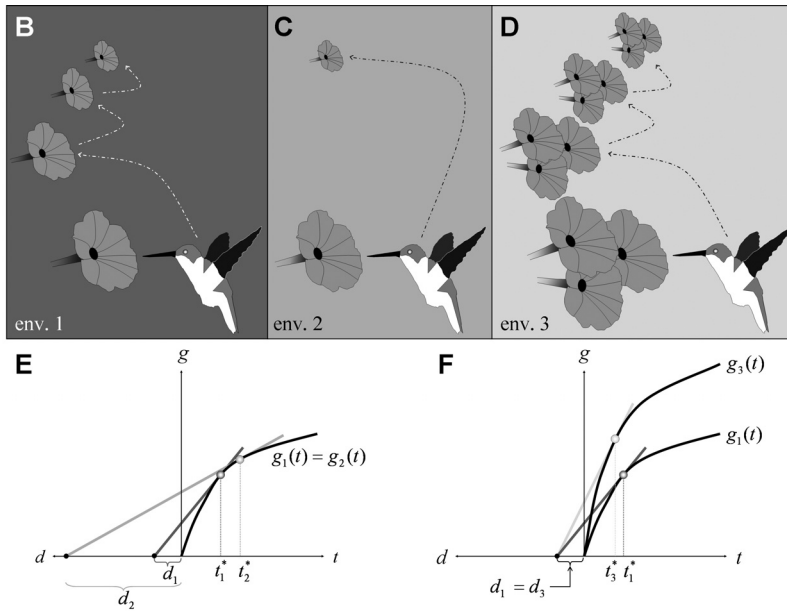

Figure 2.5 (*cont.*)

(Wang *et al.*, 1996; Abedon *et al.*, 2001; Bull *et al.*, 2004). There is empirical support for this assumption of linearity (Hutchinson and Sinsheimer, 1966; Josslin, 1970; Wang *et al.*, 1996; Wang, 2006), although other functions have also been considered (Wang *et al.*, 1996; Abedon *et al.*, 2001).

Equation 2.4 spells out the fundamental trade-off between fecundity and generation time. Each foraging phage particle can only gain more offspring at the expense of a longer generation time (Abedon *et al.*, 2003; Chapter 3). This predicament is similar to the hummingbird's problem, where energetic benefit within a patch is obtained only through costly time investment. Is there an optimal phage solution to this quandary?

When phages are rare in an environment with constant host quantity and quality, an appropriate measure of fitness is the population growth rate:

$$G = [B(L)]^{1/(D+L)} \tag{2.5}$$

where $D + L$ is the phage generation time. The latent period that maximizes G is the optimal latent period, L^*. This optimal latent period will also maximize $\ln(G)$. It can be shown that the optimal latent period is the value L^* such that the instantaneous rate of increase in the log of the burst size and the long-term rate of increase in the log of the burst size are equal. Thus, we

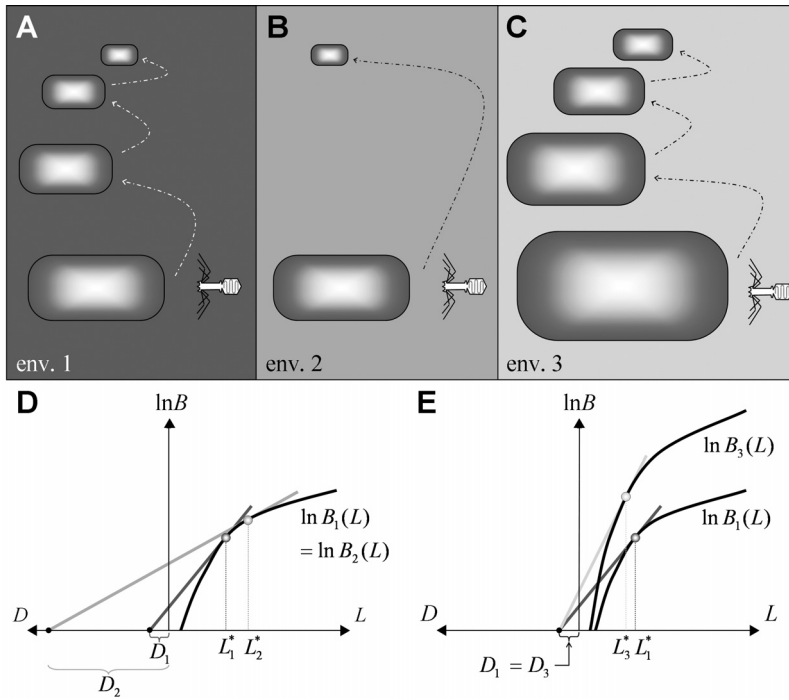

Figure 2.6 Optimal "phoraging" theory. This figure replicates Fig. 2.5 except that the forager's inter-patch dispersal time (d) is replaced by the inter-host dispersal time of the phage (D), the forager's residence time in a patch (t) is replaced by the latent period of a phage in its host (L), and the forager's energetic gain ($g(t)$) is replaced by the log of the phage burst size, $\ln[B(L)]$. As before, we consider (A) environment 1, (B) environment 2, with the same host quality, but a larger inter-host dispersal time than environment 1, and (C) environment 3, with the same inter-host dispersal time, but better host quality than environment 1. As before, we see that (D) increased inter-host travel time (e.g., by decreasing host density) favors a longer optimal latent period and (E) increased host quality favors a shorter optimal latent period. Subscripts in these figures refer to the environment to which the parameter pertains. *See color plate section.*

have a microbial marginal value theorem, where the logarithm of the burst size plays the role of the "energetic gain function," inter-host travel gives the "dispersal time," and latent period is the "residence time." Using a series of phage λ lysis-time mutants, Wang (2006) explored how fitness varied with latent period and demonstrated that an intermediate optimal latent period existed.

In Fig. 2.6, we see that we recover all the results from the hummingbird example. When dispersal period is lengthened (due to a decrease in host

quantity or adsorption rate), the optimal latent period is predicted to increase (Fig. 2.6A, B, D). When the rate of maturation for progeny phage increases (due to an increase in host quality), the optimal latent period is predicted to decrease (Fig. 2.6A, C, E). Besides several theoretical investigations into factors affecting optimal latent period (Abedon, 1989; Wang *et al.*, 1996; Abedon *et al.*, 2001; Bull *et al.*, 2004; Bull, 2006; Chapter 3), there have also been two fascinating empirical contributions. In the first study, Abedon *et al.* (2003) competed phage RB69 against a mutant with a short latent period at a variety of host densities. They found that the mutant out-competes the RB69 wild type at high host density, but the reverse occurs at low host density, consistent with the predictions (see Fig. 2.6D). In a second study, Heineman and Bull (2007) used an experimental evolution approach with phage T7. Under high host density, T7 evolved a shorter latent period (close to the predicted optimal latent period). However, these authors did not find the evolution of an optimally longer latent period when T7 was propagated under low host density (the authors attribute this result to a violation of the assumption of linear phage accumulation spelled out by Equation 2.4). Nonetheless, this work is partially consistent with earlier predictions.

2.4.3 Complications on phage optimal foraging

While there has been qualitative empirical agreement with the marginal value theorem, there are some important caveats. First, we have assumed that the dispersal time between hosts, D, is constant. Even for constant host density this is unlikely to be true (as there will be variance in waiting times between burst and infection). Using simulation models, Abedon *et al.* (2001) compared the optimal latent period of phages having a constant dispersal time between hosts to the optimal latent period of phages having an exponentially distributed dispersal time between hosts. They demonstrated that optimal latent period, especially at lower bacterial densities, is lower under the exponential model (where phage dispersal times vary). In natural phage populations, host density itself is likely to be variable, which should affect optimal latent period (Bull *et al.*, 2004). Second, we have assumed constant host quality. In natural phage populations, host quality is likely heterogeneous. This is especially true for populations of phage-sensitive and partially resistant bacteria (such as the case of phage T2 described above) and for polyvalent phages (strains that can infect multiple host species). Such heterogeneity in "patch" quality should also affect the optimal latent period (Bull, 2006).

Third, it is important to keep in mind that latent period, adsorption rate, burst size, and eclipse period are dependent not only on the phage, but also on

the host and the environment. A given phage genotype can display tremendous phenotypic plasticity. For instance, as the growth rate of the bacterial host increases, the same strain of phage T4 will increase its rate of phage production and burst size and decrease its latent and eclipse periods (Hadas *et al.*, 1997). If a single phage genotype is unable to attain the optimal latent period phenotype in every environment it encounters, then the optimal form of plasticity should depend on both the frequency of different environments and the nature of the genetic constraints (Abedon *et al.*, 2001). See Chapter 3 for discussion of additional constraints on the evolution of latent-period optimality.

Genetic constraints do not usually enter into optimality analysis, where it is assumed that selection will find a genetic path to the optimal strategy. Indeed, latent period in bacteriophages presents a remarkable test case for optimality arguments such as the marginal value theorem (Bull *et al.*, 2004). So far, the empirical results are largely consistent with the predictions of the optimality analysis. And there seem to be some solid genetic reasons for this: (1) it is genetically difficult to transition from a lytic life history to a completely different life history (such as continuous secretion from the host), and (2) mutations in phage holins (the proteins that "time" lysis) can change latent period without concomitant changes to other phage properties (Bull *et al.*, 2004; Wang, 2006). Indeed, the modularity of holins makes the latent period of lytic phages an ideal system for further investigation of predictions of optimal life-history theory.

2.5 METACOMMUNITY ECOLOGY

The models in the last three sections have assumed that interacting species encounter one another in well-mixed conditions. Such an assumption is made primarily for mathematical simplicity and tractability (Tilman and Kareiva, 1997; Dieckmann *et al.*, 2000). However, under natural conditions, this "mass action" assumption (Chapter 15) is surely misplaced. Many biological populations are broken up into subpopulations linked loosely by migration (Hanski and Gaggiotti, 2004; Holyoak *et al.*, 2005), the so-called "metapopulation." If migration is limited, then an individual will have a greater probability of interacting with a member of its own subpopulation than a random individual taken from the entire collection of subpopulations.

2.5.1 Harmony through asynchrony

Does this violation of the mass-action assumption ever matter? It turns out that it can matter significantly. For instance, consider the case in which

a subpopulation is reliably extinction-prone through overexploitation of resources. If every individual can access every subpopulation (i.e., migration is unlimited and thus the well-mixed condition is approached), then the population will crash completely. However, if individuals inhabit a metapopulation, even if subpopulations go extinct regularly, persistence at a global scale can be facilitated through asynchrony in extinctions and colonizations (Huffaker, 1958; Holyoak and Lawler, 1996; Ellner *et al.*, 2001; Bonsall *et al.*, 2002; Hanski and Gaggiotti, 2004; Holyoak *et al.*, 2005). That is, resources can be recovering in some places (after the local extinction of the exploiting subpopulation) while the organisms are temporarily thriving in other places (by depleting their local resources). A limited migration rate allows individuals to reach new resources, while avoiding complete synchrony in resource use – and this can allow persistence.

Several researchers have paid particular attention to the behavior of "metacommunities" (Holyoak *et al.*, 2005), a set of interacting species distributed in spatially isolated patches connected by migration of the community members. In a now famous experiment, Huffaker (1958) embedded communities of predatory mites and phytophagous mites (the prey) within a network of oranges. By manipulating the exposed fruit surface and distribution of fruits, Huffaker demonstrated that increased patchiness can promote coexistence of predators and their prey. In essence, the prey is the "resource" for the predator, and limitations in migration (with the resulting asynchrony) can help maintain diversity in the system. This finding has now been confirmed in other laboratory studies as well (Holyoak and Lawler, 1996; Bonsall *et al.*, 2002).

2.5.2 Metacommunities of bacteria and phages

Recently, a similar result was described in bacterium–phage metacommunities (Kerr *et al.*, 2006). These authors embedded phage T4 and *E. coli* B in 96-well microtiter plates and used a high-throughput liquid-handling robot to perform serial transfers of the entire metacommunity. The robot was also used to execute microbial migrations between subpopulations. While the bacteria and phages could not coexist within a single microtiter well (a subpopulation), these authors demonstrated that coexistence was possible at the level of the metacommunity (Fig. 2.7). The precise structure of the metacommunity (e.g., the rate and pattern of migration) turns out to influence evolutionary dynamics within this bacteria–phage system. Such evolutionary dynamics are the subject of the next section.

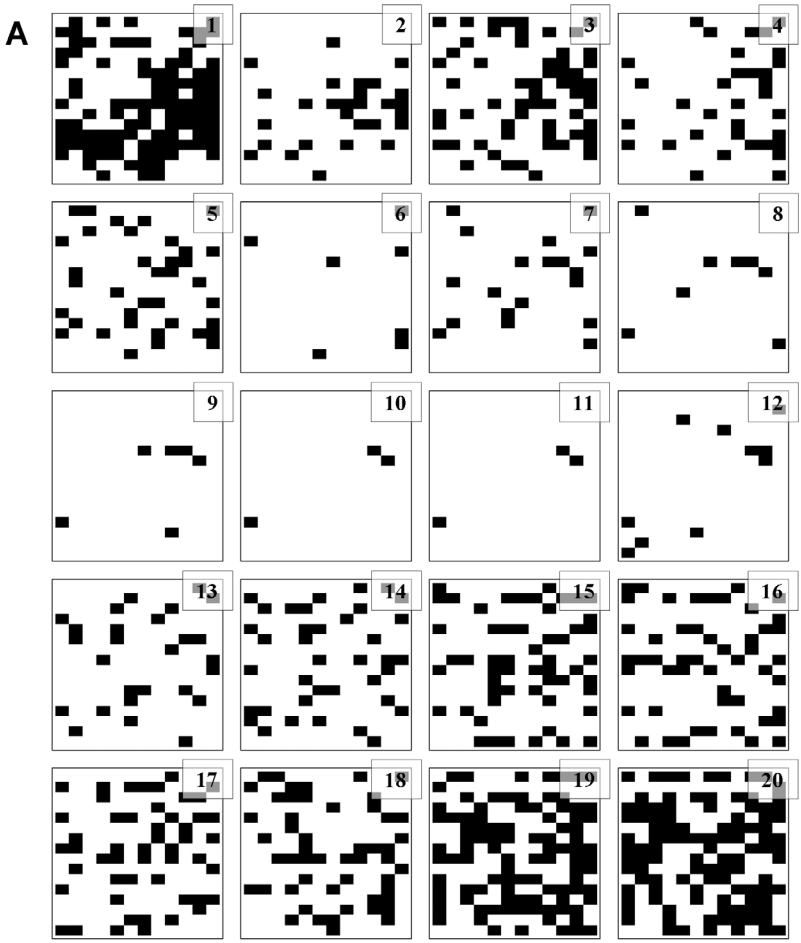

Figure 2.7 A bacterium–phage metacommunity. (A) Each plot gives the spatial distribution of bacteria-filled wells (black squares) in a 192-well metacommunity at different transfers (the numbers in the upper right of each plot) throughout the serial propagation. The white squares are either medium-filled or phage-filled. The migration pattern applied at each transfer (termed "unrestricted" in Kerr *et al.*, 2006) allowed any well to receive microbial migrants, from one of any other well in the metacommunity, with 45% probability. The number of bacteria-filled wells approached low levels during transfers 8 through 12. However, the bacterial population rebounded shortly thereafter. (B) This graph shows the bacterial (squares) and phage (circles) densities corresponding to the spatial distributions shown in part A. Even though bacteria and phages could not coexist within a single well, bacteria and phages are maintained in the metacommunity.

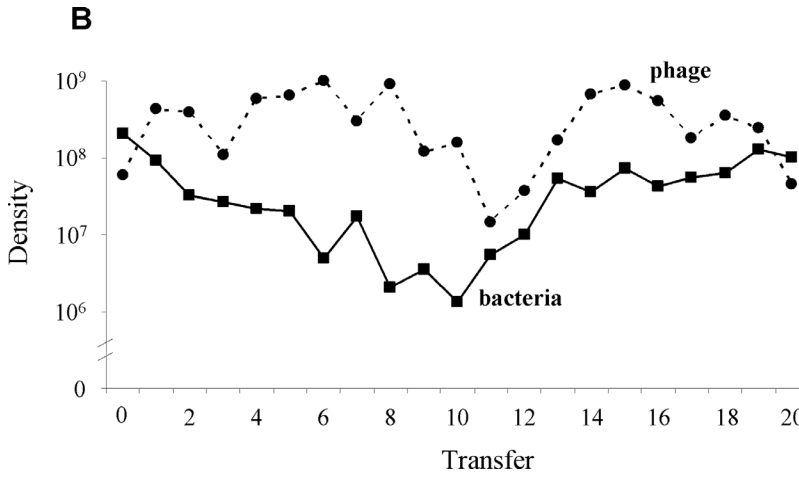

Figure 2.7 (*cont.*)

2.6 EVOLUTIONARY ECOLOGY

How ecological factors shape and are shaped by evolutionary change forms the subject matter of evolutionary ecology. A major challenge within this field is understanding the adaptive significance of the very nature of ecological interactions. Victim–exploiter relationships have an intrinsically antagonistic component (i.e., exploiters damage their victims for personal gain). However, how damaging should an exploiter be? Can exploitative restraint ever be evolutionarily favored? What are the ecological circumstances that select for different ways to use victims? These questions illustrate that ecological interactions (such as those between predators and prey, parasites and hosts, herbivores and plants, etc.) are not static, but have an evolutionary history. It turns out that factors such as migratory range of exploiters and their mode of travel between victims can profoundly influence the nature of these interactions.

2.6.1 Phage productivity versus competitive ability

In the aforementioned metacommunity study of Kerr *et al.* (2006), treatments consisted of different migration patterns within the collection of subpopulations. One treatment restricted migration of microbes to neighboring microtiter wells; whereas in another treatment, migration was unrestricted (i.e., microbes could move from any subpopulation to any other subpopulation with some probability). After several transfers, these authors measured

phage productivity (the number of phages produced per parent in a well with host bacteria) and competitive ability (fitness of the phages relative to a marked strain when exposed to shared hosts). They discovered that phages in the restricted treatment evolved high productivity and low competitive ability. On the other hand, phages in the unrestricted treatment evolved low productivity and high competitive ability. In fact, a negative correlation was found between productivity and competitive ability overall, suggesting a trade-off. See Chapter 3 for additional consideration of phage trade-offs between productivity and competitive ability, and Chapter 6 for a more general discussion of trade-offs in the evolution of microbial systems (e.g., in the context of phage host range).

2.6.2 Tragedy of the commons

The results of Kerr *et al.* (2006) suggested the possibility of a 'tragedy of the commons' (Hardin, 1968). This phrase pertains to scenarios in which multiple users exploit the same resource. Although unrestrained users may out-compete restrained users, the rise of unrestrained users may cause the resource (the 'commons') to be overexploited, lowering overall user productivity (the 'tragedy'). Within a subpopulation, resident phages exploit bacterial resources. Prudent use of this resource (due to longer latent period or slower adsorption) leads to higher productivity of the phages (Chapter 3). The 'tragedy of the commons' is that rapacious types lower overall productivity as they displace more restrained competitors. Abedon *et al.* (2003) demonstrated that rapacious variants of phage RB69 can out-compete their prudent ancestors by limiting future access to the host through their own rapid consumption. Further, these authors found that when grown in pure culture, the prudent ancestor was more productive than its rapacious descendant. The findings of Abedon *et al.* (2003) are consistent with the trade-off postulated by Kerr *et al.* (2006) and the potential for a 'tragedy of the commons' (Chapter 3).

2.6.3 Evolution of restraint given spatial structure

Why might different patterns of migration favor different resolutions of this tragedy? Within any subpopulation, rapacious phage mutants always outcompete their prudent ancestors, but these superior competitors are less productive and therefore more likely to go extinct (given periodic dilution). Low productivity is the Achilles heel of the rapacious phage, so its success hinges

on gaining sufficient access to fresh hosts. Unrestricted migration ensures that rapacious phages have continual access to hosts. Furthermore, unrestricted migratory patterns increase the probability of mixing phage types, which favors the rapacious competitor. In contrast, restricted migration lowers the likelihood that different phage types mix and lowers the accessibility of fresh hosts. Thus, rapacious phages are more vulnerable to extinction in the restricted treatment, leaving the metacommunity relatively prudent by default (Kerr *et al.*, 2006).

In the 'tragedy of the commons' scenario, the trade-off between competitive ability and productivity is key to understanding the evolution of restraint in one of the aforementioned treatments. Trade-offs are a general theme in evolutionary ecology (Bohannan *et al.*, 2002). For example, trade-offs figure prominently in the literature concerning the evolution of pathogen virulence (Bull *et al.*, 1991; Herre, 1993; Ebert, 1994; Nowak and May, 1994; Lipsitch *et al.*, 1995; Boots and Sasaki, 1999; O'Keefe and Antonovics, 2002; Galvani, 2003; Thrall and Burdon, 2003). In this work, virulence is assumed to covary with pathogen transmission (Herre, 1993; Ebert, 1994; Nowak and May, 1994; Lipsitch *et al.*, 1995; Boots and Sasaki, 1999; Galvani, 2003) or competitive ability (Nowak and May, 1994; Keeling, 2000). Assuming these or similar trade-offs, many researchers have investigated the role of host population structure on the evolution of pathogen strategy (Bull *et al.*, 1991; Herre, 1993; Lipsitch *et al.*, 1995; Boots and Sasaki, 1999; Keeling, 2000; O'Keefe and Antonovics, 2002; Galvani, 2003; Thrall and Burdon, 2003). There are theoretical and experimental results that suggest that as populations become less structured, virulence in pathogens is favored (Bull *et al.*, 1991; Boots and Sasaki, 1999; Keeling, 2000; O'Keefe and Antonovics, 2002; Galvani, 2003). See Chapters 4 and 16 for additional consideration of the phage ecology of spatially structured habitats, and Chapter 3 for an additional consideration of phage virulence.

2.6.4 Evolution of restraint with vertical transmission

Using phage f1 and *E. coli* K12, Bull *et al.* (1991) demonstrated that horizontal transmission of the phage (as would occur in a less structured population) selected for variants that were relatively damaging to their hosts (i.e., highly virulent); whereas vertical transmission (as would occur in a more structured population) selected for avirulent phage. Furthermore, using the same system, Messenger *et al.* (1999) demonstrated that a trade-off existed between virulence and reproductive output. The level of virulence that evolved

depended on the ratio of vertical to horizontal transmission, with viral lines evolving higher virulence and higher reproductive output when the ratio was low. The 'tragedy of the commons' illustrates that strategies yielding short-term gain (high viral reproductive output or high viral competitive ability) may be disastrous for long-term success. When hosts are unlimited (i.e., horizontal transmission readily occurs), then maximizing short-term gain can be optimal; whereas when access to fresh hosts is limited (i.e., vertical transmission is more prevalent), then investment in long-term gain pays off and avirulent strategies become more favorable.

2.6.5 Linking evolution and ecology

As the topic of pathogen virulence illustrates, the synergy of evolutionary and ecological approaches can provide useful tools for a deeper understanding of biological systems. This synergy is particularly appropriate for the study of bacteriophages because of their short generation times, large population sizes, heterogeneous environments, and diverse set of biotic interactions. Complementarily, bacteriophage systems form ideal models for exploring the intersection of ecology and evolution: such systems not only demonstrate basic principles of ecology, but enlarge our ecological perspective to include evolutionary dynamics.

For example, the evolution of resistant hosts and host-range phage mutants within experimental microbial communities shows that the study of community structure is enriched by a consideration of *de novo* evolutionary contributions. As another example, optimal life-history theory assumes that evolutionary change will produce the strategy most appropriate for a set of ecological conditions; thus, how phage latent period changes in response to host quantity and quality is an issue well-suited for evolutionary ecology. Similarly, the patterns of migration in metacommunities not only influence the abundances of phages and bacteria, but also their evolutionary trajectories. Overall, research on bacteriophages exemplifies how evolutionary approaches inform various fields within ecology, including community ecology, behavioral ecology, and metapopulation ecology.

2.7 FUTURE DIRECTIONS

In this chapter we have highlighted only a few of the ecological concepts that can be fruitfully explored with, and applied to, phages and bacteria, using studies of coliphages and *E. coli* as examples. These examples demonstrate

that ecological concepts can be very useful for increasing our understanding of phages and bacteria, and that in turn phages and bacteria can be very powerful tools for exploring such concepts. Our examples, and indeed most studies of the interactions between phages and bacteria, are of relatively simple communities maintained in homogeneous laboratory environments. The most exciting future directions for such research require the relaxation of these constraints.

Phages and bacteria are ideal for exploring the ecological implications of spatial structure and environmental heterogeneity. Indeed, several recent studies have begun to do exactly this (Brockhurst *et al.*, 2003, 2006; Forde *et al.*, 2004; Kerr *et al.*, 2006; Chapters 3, 4, and 16). Promising areas of focus include the effects of heterogeneous environments on stability, diversity, and evolution within communities consisting of bacteria and phages. In order to explore such topics, microbial community modules could be embedded within metapopulation apparatus (e.g., microtiter plates) or spatially continuous surfaces (e.g., agar-filled Petri dishes; Chapters 4 and 16). Such setups could also be employed to explore the optimal latent period of lytic phages in environments with spatially heterogeneous host density and quality.

Most studies of interactions between phages and bacteria have utilized only one phage type. However, ecological theory predicts that population dynamics, the opportunities for coexistence, and the trajectory of evolution can be very different when multiple predator types, rather than just one, are present (Weitz *et al.*, 2005). It would be interesting to explore the potential for coexistence of phages with different life histories (e.g., obligately lytic and temperate phages) living with a shared pool of hosts. Also, by using phages with different life histories, researchers could explore the evolutionary effects of one phage on another through exploitative competition for hosts. The vast majority of ecological studies of phages and bacteria have focused on obligately lytic phages, i.e., phages that invariably kill their hosts after a relatively short latent period (Chapter 1). However, phages exhibit a range of exploitative lifestyles, including those displayed by temperate phages (which can incorporate into their host's genome for a variable time period, before re-emerging and killing their host; Chapter 5) and filamentous phages (which can release progeny from their hosts without host death). Utilizing the full range of phage lifestyles will permit the application of a wide range of ecological concepts to phages and bacteria, and allow the exploration using laboratory communities of a much longer list of ecological questions. For example, temperate and filamentous phages are closer to traditional parasites than are obligately lytic phages, which are most precisely parasitoids.

Lastly, future research will ideally begin to bridge the gap between ecological studies of phages and bacteria in the laboratory and recent surveys of viruses in natural environments (e.g., Chapters 10 and 11). The study of viruses in nature (the majority of which appear to be bacteriophages) has grown rapidly in recent years, due to methodological developments that permit the quantitative study of viral populations without requiring laboratory culture (Fuhrman, 1999; Chapter 10). Future research could assess the relevance of experimental results with simple phage modules – the topics discussed in this chapter – to the complex webs of natural phage communities.

2.8 APPENDIX

Here we explore the chemostat model of Bohannan and Lenski (1997). They write the following system of differential equations describing the dynamics for the concentration of resource (C), the concentration of the bacterial prey (N), and the concentration of the phage predator (P):

$$dC/dt = (C_0 - C) \cdot \omega - \varepsilon \cdot N \cdot \Psi \cdot C/(K + C) \tag{2.6}$$

$$dN/dt = N \cdot \Psi \cdot C/(K + C) - \boldsymbol{\alpha} \cdot N \cdot P - \omega \cdot N \tag{2.7}$$

$$dP/dt = B \cdot e^{-L \cdot \omega} \boldsymbol{\alpha} \cdot N' \cdot P' - \boldsymbol{\alpha} \cdot N \cdot P - \omega \cdot P \tag{2.8}$$

where C_0 is the concentration of glucose feeding into the chemostat, ω is the flow rate through the chemostat, ϵ is the reciprocal yield of the bacteria, Ψ is the maximal bacterial growth rate, K is the resource concentration at which the bacteria grow at one-half their maximal rate, α is the adsorption rate of phage, B is the burst size, and L is the latent period. All primed variables are evaluated L time units ago, e.g., $N' = N(t - L)$. As a baseline, we use the following values: $C_0 = 0.25\ \mu g\ mL^{-1}$, $\omega = 0.2\ h^{-1}$, $\varepsilon = 2 \times 10^{-6}\ \mu g$, $\Psi = 0.7726\ h^{-1}$, $K = 0.0727\ \mu g\ mL^{-1}$, $\alpha = 3 \times 10^{-7}\ mL\ h^{-1}$, $B = 80$, and $L = 0.6$ h.

We manipulate the productivity of the system by altering C_0. In Fig. 2.8A we see that the predator–prey cycles increase in amplitude as the system is enriched. We manipulate the negative density dependence of the prey by altering K. In Fig. 2.8B we see that negative density dependence has a stabilizing effect (as K is increased, the amplitude of the cycles diminish). We manipulate the time lag in the system by altering the latent period (L). In Fig. 2.8C, we see that increasing the latent period slightly destabilizes the system. As we discuss in Section 2.4, phage burst size is positively correlated with latent period; we will assume $B = R \cdot (L - E)$. If we perform the same perturbations in latent period as shown in Fig. 2.8C (but allow burst size to

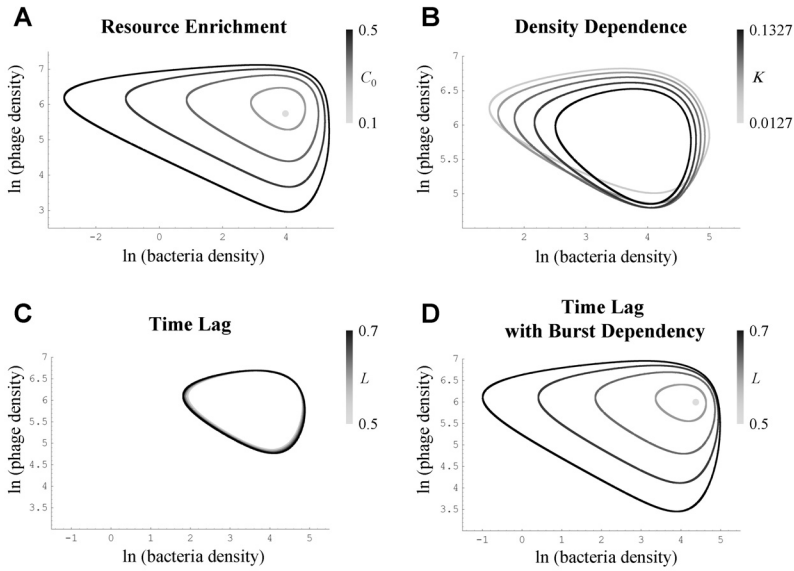

Figure 2.8 Predicted chemostat limit cycles. Unless otherwise noted, parameter values are as presented in Section 2.8. (A) We varied glucose reservoir concentration C_0 from 0.1 μg L^{-1} to 0.5 μg L^{-1}. Resource enrichment destabilizes the system. (B) Letting $C_0 =$ 0.25 μg L^{-1}, $L = 0.6$ h, and $B = 80$, we varied the half-saturation constant K from 0.0127 μg L^{-1} to 0.1327 μg L^{-1}. Negative density dependence (achieved through larger K values) stabilizes the system. (C) Letting $C_0 = 0.25$ μg L^{-1}, $K = 0.0727$ μg L^{-1}, and $B = 80$, we varied the latent period L from 0.5 h to 0.7 h. These increases in time lag slightly destabilize the system. (D) Letting $C_0 = 0.25$ μg L^{-1} and $K = 0.0727$ μg L^{-1}, we varied the latent period L from 0.5 h to 0.7 h, but we let the burst size be a linear function of the latent period (see Section 2.4): $B = R \cdot (L - E)$. We set $E = 0.4333$ h (Abedon *et al.*, 2001) and $R = 480\ h^{-1}$: this rate of phage increase was chosen so that the burst size function would intersect the point $(L, B) = (0.6, 80)$. Compared to the case where burst size is held constant (C), an increase in latent period with a simultaneous increase in burst size dramatically destabilizes the system.

change according to the above function with eclipse period, $E = 0.4333$ h, and the rate of progeny production, $R = 480\ h^{-1}$) then we see that the system is substantially destabilized by increases in the latent period (Fig. 2.8D).

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