

Paper IV



Trade-offs and coexistence in microbial microcosms

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Abstract

Trade-offs among the abilities of organisms to respond to different environmental factors are often assumed to play a major role in the coexistence of species. There has been extensive theoretical study of the role of such trade-offs in ecological communities but it has proven difficult to study such trade-offs experimentally. Microorganisms are ideal model systems with which to experimentally study the causes and consequences of ecological trade-offs. In model communities of *E. coli* B and T-type bacteriophage, a trade-off in *E. coli* between resistance to bacteriophage and competitive ability is often observed. This trade-off can allow the coexistence of different ecological types of *E. coli*. The magnitude of this trade-off affects, in predictable ways, the structure, dynamics and response to environmental change of these communities. Genetic factors, environmental factors, and gene-by-environment interactions determine the magnitude of this trade-off. Environmental control of the magnitude of trade-offs represents one avenue by which environmental change can alter community properties such as invasability, stability and coexistence.

Introduction

The existence of so great a diversity of species on Earth remains a mystery, ... the answer may lie in quantifying the trade-offs that organisms face in dealing with the constraints of their environment.

David Tilman (2000)

The most striking aspect of life on Earth is its vast diversity. This is especially true for microorganisms, which likely represent most of the earth's biodiversity (Whitman et al. 1998) and may consist of millions of species (Dykhuizen 1998). How is this diversity maintained in nature? What allows all of these species to coexist? Most theories describing the coexistence of species assume trade-offs among the abilities of organisms to respond to the different factors that constrain their fitness and abundance (Tilman 2000). A trade-off occurs whenever a trait that confers an advantage for performing one function simultaneously confers a disadvantage for performing another function. Such trade-offs are likely because there are limitations on

how energy and other cellular resources are allocated to different functions, and because of physical and chemical constraints. For example, a trait that increases an organism's ability to obtain and utilize a particular energy source may simultaneously reduce its ability to obtain and utilize other energy sources. Such a trade-off could allow the coexistence of a number of types of organisms, each specialized in the use of a particular energy source. Coexistence is possible because the trade-off results in organisms with different growth requirements for and different impacts on the environment. These differences can prevent competitive exclusion and thus allow coexistence.

There are many potential trade-offs that could allow the coexistence of organisms, including trade-offs between competitive ability and dispersal ability, between abilities to compete for alternative resources, between competitive ability and susceptibility to predation, herbivory or disease, and between competitive abilities in constant and variable environments (Grover 1997; Dykhuizen 1998; Travisano & Rainey 2000;

Huisman and Wessing 2001; Huisman et al. 2001). There has been extensive theoretical study of the role of such trade-offs in ecological communities but it has proven difficult to study such trade-offs experimentally. This is due in part to difficulty directly observing the existence of trade-offs (Bergelson 1994). For instance, the trade-off might be too small to be easily detected (Mittra & Bhatia 1982), even though it may be of sufficient magnitude to substantially influence the coexistence of organisms. Also, multiple observations of different organisms in different contexts can seem contradictory given that the existence and magnitude of a trade-off may vary with the environment and genotype (Bohannon et al. 1999).

The most successful studies of trade-offs have been in laboratory systems (e.g., Lenski 1988a; Bergelson et al. 1996; Grover 1997; Kraaijeveld & Godfray 1997; Bohannon et al. 1999), especially those consisting of microorganisms (e.g., Lenski 1988a; Bohannon et al. 1999; Feldgarden & Riley 1999; Levin et al. 2000). In laboratory communities of microorganisms it is possible to directly observe a trade-off, to determine the influence of genotype and environment on the magnitude of a trade-off and to study the consequences of these effects on community-level processes such as the coexistence of organisms. In this paper, we discuss the results of several recent and ongoing experiments using *E. coli* B and T-type coliphage as a model system to study the causes and consequences of ecological trade-offs.

Overview of experimental system

The trade-off between competitive ability and resistance to predators forms the basis for predator-mediated coexistence of species and has received a great deal of study, both theoretical and empirical (Paine 1966; Lubchenco 1978; Lenski 1988b; Leibold 1996; Grover 1997; Kraaijeveld & Godfray 1997; Bohannon et al. 1999). In addition, this trade-off forms the basis for many aspects of predator-prey coevolution (Thompson 1989; Dieckmann & Doebeli 1999). However, the existence of this trade-off and its importance have been difficult to observe (e.g., Simms 1992). Laboratory communities of phage and bacteria offer the ability to experimentally study the trade-off between resistance to predators and competitive ability (reviewed in Bohannon and Lenski 2000b).

Viruses that infect bacteria (bacteriophage) are ubiquitous predators of bacteria and have the potential

for promoting the coexistence of bacterial populations (Fuhrman 1999; Wommack & Colwell 2000). There are two general classes of bacteriophage: temperate phage (i.e., phage that can exist in a quiescent state in bacterial cells without killing the cell) and lytic phage (i.e., phage that invariably kill the host cell following infection and phage reproduction) (Lenski 1988c). Most studies that have used bacteriophage as predators in laboratory communities have used lytic phage. The lytic T-series bacteriophage (T4, T2, T7 and their relatives) are voracious consumers of the bacterium *E. coli* and are the most commonly used model predators (Chao et al. 1977; Levin et al. 1977; Lenski & Levin 1985; Schrag & Mittler 1996; Bohannon & Lenski 1997, 1999, 2000), although virulent (lytic) mutants of phage lambda have also been used (Horne 1970; Schrag & Mittler 1996).

A brief overview of T-phage/*E. coli* biology is provided below; for a more complete description see Bohannon & Lenski (2000b). The life cycle of a T-type phage begins with its adsorption to the surface of a bacterium. Adsorption of T-type phage to the bacterial cell surface occurs through a highly specific interaction between the phage's tail fibers and a receptor molecule on the cell surface (Goldberg et al. 1994). Following adsorption, the phage DNA is injected into the bacterial cell. After a latent period during which phage particles are assembled, the bacterial cell is destroyed and the phage progeny are released.

Mutant bacteria that are invulnerable to phage infection have been reported for all of the T-type phage (Figure 1A; Lenski 1988c). Most phage-resistant mutants achieve their resistance through the loss or modification of the receptor molecule to which the phage initially binds. These mutations often simultaneously reduce the cell's competitive ability (Figure 1B) because the receptor molecules are also involved in aspects of bacterial metabolism such as the uptake of nutrients (Lenski 1988a). Despite this trade-off, it is commonly observed that phage-resistant mutants quickly evolve and invade most laboratory communities of T-type phage and *E. coli* (Figure 1A; Lenski 1988c). The trade-off between resistance and competitiveness can allow the stable coexistence of sensitive and resistant *E. coli* when phage are present (Lenski 1988c).

It is likely that resistance to phage varies quantitatively in *E. coli*, with some mutants completely resistant to phage attack and others partially resistant. However, there has been little study of quantitative resistance in *E. coli*. Most of the research concerning

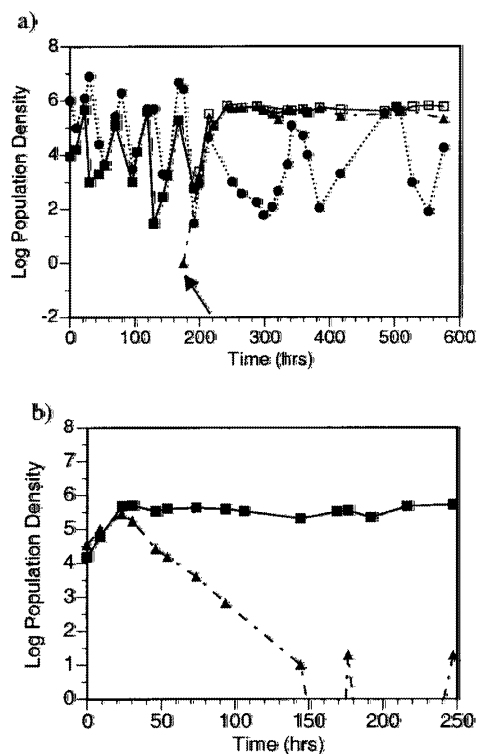


Figure 1. (a) Invasion of a chemostat community of phage T4 and phage-sensitive *E. coli* by phage-resistant mutants. The population densities (viruses per ml or bacteria per ml) of T4-sensitive *E. coli* (squares), T4-resistant *E. coli* (triangles), and bacteriophage T4 (circles) have been \log_{10} -transformed. The arrow indicates when the mutant was first detected. Once the resistant mutant invaded the chemostats, it became impossible to track sensitive *E. coli* directly; only the sum of the sensitive and resistant *E. coli* (open squares) was quantified. [From Bohannan & Lenski (1997, Figure 3B), with permission of *Ecology*, © 1997 by the Ecological Society of America]. (b) Population dynamics of T4-sensitive (squares) and T4-resistant (triangles) *E. coli* in a representative chemostat lacking phage. The decline of the resistant population is due to the trade-off between resistance and competitiveness. The population densities (bacteria per ml) have been \log_{10} -transformed. [From Bohannan & Lenski (2000, Figure 6F), with permission of *Ecology Letters*, © 2000 by Blackwell Scientific].

quantitative or partial phage resistance has been done using *E. coli* and phage T2. This is primarily because *E. coli* mutants that are partially resistant to bacteriophage T2 have the correlated trait of complete resistance to bacteriophage T4 (Lenski 1984). This relationship allows the partially resistant mutants to be easily obtained (by selecting for T4-resistant mutants) and

easily tracked in laboratory microcosms (by screening for T4-resistant bacteria). A trade-off between competitive ability and partial resistance to T2 has been demonstrated in *E. coli* growing in glucose-limited environments (Lenski & Levin 1985; Lenski 1988a).

We used microcosms of *E. coli* and T-type phage to study the causes and consequences of ecological trade-offs, focusing on the trade-off between resistance to phage and competitive ability in *E. coli* as an example. The microcosms were maintained using two different culture systems: continuous culture and batch culture. Our continuous culture microcosms were maintained in 30 ml of media in custom-made chemostats, as described elsewhere (Bohannan & Lenski 1997). Our batch culture microcosms were maintained in 10 ml of media in 50 ml erlenmeyer flasks, transferred at intervals of 24 h (Lenski et al. 1991). Davis minimal medium supplemented with a carbon source (glucose, trehalose, or maltose) was used as the growth medium in all microcosms (Carlton & Brown 1981). The populations were regularly sampled and enumerated on Davis minimal plates (Carlton & Brown 1981).

Experimental findings

The magnitude of the trade-off varies with genotype and environment

Ecological trade-offs can potentially allow competing populations to coexist. However, whether or not coexistence actually occurs is dependent in part on the magnitude of a trade-off (Bohannan & Lenski 2000b). The magnitude of the trade-off is the degree to which improvement in one trait results in detriment to another trait. For example, resistance to phage is often associated with a decrease in competitive ability. The degree to which competitive ability is decreased is the magnitude of the trade-off, which is also referred to as the 'cost' of resistance. What determines the magnitude of an ecological trade-off? In the most fundamental sense, the magnitude of a trade-off is determined by properties of individuals and how these properties interact with the environment. For example, different mutations that confer equally complete phage-resistance can vary in the magnitude of their trade-off with competitiveness (Lenski 1988a; Bohannan & Lenski 2000b). The magnitude of the trade-off can vary with the degree of cross-resistance to other phage (Bohannan & Lenski 2000b). Resistance mutations can also interact with other genes to

Table 1. Analysis of variance among 5 T4-resistant genotypes for relative fitness in DM supplemented with either 25 mg or 1 g of either glucose or maltose per liter.

Source	SS	df	MS	F	P
Sugar	0.022	1	0.022	10.198	0.003**
Concentration	0.016	1	0.016	7.610	0.009**
Block	0.001	1	0.001	0.514	0.478
Sugar \times concentration	0.004	1	0.004	2.113	0.155
Error	0.074	35	0.002		

alter the magnitude of the trade-off. For example, Bohannan et al. (1999) observed that it was less costly to *E. coli* to be resistant to both T4 and the phage lambda than would be expected from the costs associated with each mutation alone. However, the extent of cost savings depended on the environment in which fitness was measured as well as subtle differences among the mutations. Modifier alleles also interact with resistance alleles to mitigate costs. Lenski (1988b) observed that the cost of T4-resistance declined by almost 50% during 400 generations of evolution in absence of phage, owing to further genetic substitutions that compensated for the maladaptive side-effects associated with T4 resistance. The magnitude of the trade-off can also be altered by interactions between resistance alleles and the environment. For example, the magnitude of the trade-off between resistance to phage T4 and competitive ability is different when glucose is the growth-limiting resource than when the carbohydrate trehalose is limiting (Bohannan et al. 1999). The concentration of the resource can also alter the trade-off. For example, in a preliminary analysis of this trade-off in two different single-limiting resource environments (glucose- and maltose-limited minimal medium) at two concentrations (25 mg/l and 1000 mg/l) we observed statistically significant effects due to both sugar identity and concentration (Table 1). The cost of being resistant was higher at lower nutrient concentrations and when the growth-limiting nutrient was glucose.

Other aspects of the environment can also affect the magnitude of the trade-off. We have observed that the magnitude of the trade-off between phage T4-resistance and competitive ability for glucose may vary between batch culture and chemostat culture, even if the growth conditions are otherwise identical (Figure 2). The magnitude of this trade-off tends to be higher in chemostat culture, suggesting that life his-

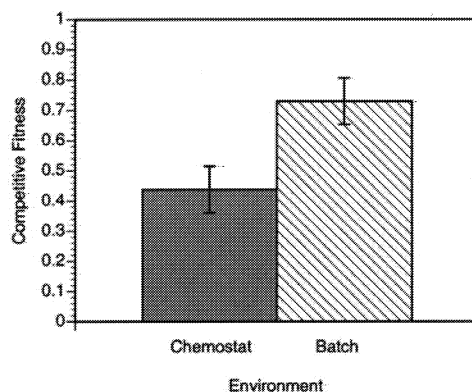


Figure 2. Relative fitness of 6 T4-resistant mutants of *E. coli* assayed in both batch and chemostat culture. Batch assays were performed as described in Lenski et al. (1991) and chemostat assays were performed as described in Bohannan & Lenski (1997). Each assay was replicated at least twice. Each bar depicts the grand mean of all replicates of all assays in the given environment (batch or chemostat). Error bars indicate standard errors of the mean. The difference between the grand means is significant ($df=5$, $t = 4.1932$, $P = 0.0085$).

tory traits that are particularly important in chemostat culture (such as growth at low resource concentrations) are more affected by mutations conferring phage resistance than those particularly important in batch culture (such as growth at high concentrations of resource). This is consistent with our observations of a higher cost at lower nutrient concentrations, as described above. Finally, there is also evidence that the cost of T4-resistance may depend on temperature (M. Travisano, pers. comm.).

The trade-off has direct consequences

The magnitude of a trade-off can have both direct and indirect effects on a community. By understanding the physiological, genetic and environmental influences on this trade-off we can predict how the trade-off will affect community properties such as invasibility, community structure and the coexistence of populations. For example, the magnitude of the trade-off is predicted to directly affect the likelihood that a phage-resistant mutant can invade a community of phage and sensitive bacteria. The higher the competitive 'cost' of being resistant (i.e., the larger the trade-off), the higher the probability of extinction of a resistant mutant in laboratory culture (Figure 3; Bohannan & Lenski 2000b). When a resistant mutant can successfully invade a resident community its rate of invasion

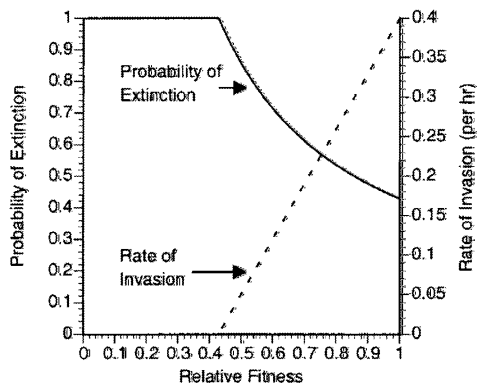


Figure 3. Predicted effect of the relative fitness (i.e., $1 - [\text{cost of resistance}]$) of a phage-resistant strain on the strain's probability of extinction (solid line) and rate of invasion (dashed line). Modified from Bohannan & Lenski (2000b).

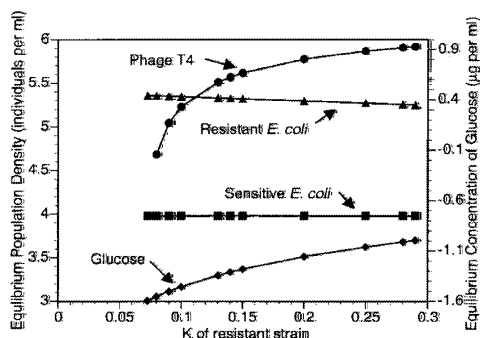


Figure 4. Effect of K on the equilibrium densities of phage-sensitive *E. coli* (squares), phage-resistant *E. coli* (triangles), T4 phage (circles) and glucose (diamonds). K is the concentration of glucose at which the bacteria grow at one half of their maximum growth rate. K is an important component of the cost of resistance; the higher the K , the larger the cost. The predictions are produced using the mathematical models described in Bohannan & Lenski (1999).

is also predicted to be a function of the magnitude of the trade-off (Figure 3; Bohannan & Lenski 2000b). Once a resistant bacteria population has successfully invaded a community, the magnitude of the trade-off between phage resistance and competitive ability can directly influence the average density of the growth-limiting resource (Figure 4; Levin et al. 1977). If the resistant population is not completely resistant, the magnitude of the trade-off will also directly affect the density of phage (Levin et al. 1977). Thus, the fate of each resistance mutation, and its direct effects on community properties, depends on the corresponding decrease in competitive ability, which in turn depends

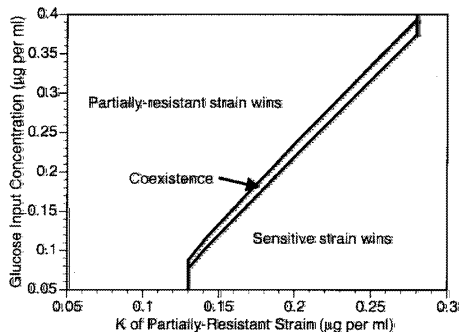


Figure 5. Effect of K and glucose input concentration on the coexistence of phage-sensitive *E. coli*, partially phage-resistant *E. coli* and T2 phage in glucose-limited chemostats (K is defined in the legend for Figure 4). The predictions are produced using the mathematical models described in Bohannan & Lenski (2000a).

on the exact genetic mutation, its physiological effects and its interactions with other genes and the abiotic environment (see above).

The trade-off can also have indirect consequences

By directly affecting the biotic and abiotic environment, the magnitude of a trade-off can have important, and predictable, environmentally-mediated indirect effects on a community. For example, if a resistant population is completely resistant, the magnitude of the trade-off between phage-resistance and competitive ability is predicted to indirectly alter the average density of phage, even though the resistant population does not directly interact with the phage (Figure 4; see also Levin et al. 1977). This occurs because the trade-off determines the average density of the growth-limiting resource, which determines the growth rate of sensitive *E. coli*, which determines the average population density of phage. Preliminary observations of chemostat communities of phage T4, sensitive *E. coli*, and T4-resistant *E. coli* with different costs of resistance support these predictions (Sandvik & Bohannan, unpubl.).

If a phage-resistant mutant does not pay a fitness cost for being completely resistant (i.e., there is no trade-off between predator resistance and competitive ability) the mutant will lower the resource density such that it will exclude the sensitive strain indirectly via resource competition, and ultimately exclude the phage as well (Lenski & Levin 1985).

If the resistant mutant is not completely resistant to phage attack (i.e., it is partially-resistant), then

it is also possible for the sensitive and resistant *E. coli* to interact negatively via their shared phage, a process known as apparent competition (Holt, 1977; Bohannan & Lenski 2000a). Apparent competition occurs because the partially resistant bacteria on average support a larger phage population than the sensitive bacterial population, and thereby can intensify predation pressure on the sensitive population (Levin et al. 1977; Lenski 1984). The magnitude of the trade-off between phage-resistance and competitive ability is predicted to determine the likelihood and rate of indirect exclusion via resource and apparent competition (Figure 5), although this has not yet been tested experimentally.

The trade-off can affect the response of a community to environmental change

An understanding of the nature of trade-offs, the genetic and environmental influences on their magnitude and their direct and indirect ecological effects allows us to predict the response of a community to environmental change. For example, if an environment is perturbed by increasing the input of energy or resources, then a chemostat community with only sensitive bacteria and phage responds with a large increase in the phage population, a small increase in the bacteria, and a reduction in the dynamic stability of both populations (Bohannan & Lenski 1997). By contrast, a chemostat community that also contains completely resistant bacteria responds to the same increase in productivity with an increase in the resistant population, no change in the average density of the phage or sensitive bacteria, and reduced stability of the sensitive bacteria and phage populations but increased stability of the resistant population (Bohannan & Lenski 1999). The degree to which the resistant population alters the response of a community to such change is a function of the magnitude of the trade-off between resistance and competitive ability. For example, if the magnitude is large (i.e., the resistant cells pay a large competitive cost to be resistant) then the effect of the resistant population on the dynamics of the resource is predicted to be diminished such that the populations of phage and sensitive bacteria are more severely destabilized by an increase in resources. Under such a scenario, the resistant population is also predicted to be stabilized to a lesser degree by the increase in resources.

If the resistant population is only partially resistant, then an increase in resources can result in the exclusion of the sensitive population via appar-

ent competition (see above). However, this outcome is dependent on the magnitude of the trade-off between resistance and competitive ability. An increase in the magnitude could result in coexistence rather than exclusion of the sensitive population, or even exclusion of the resistant population instead (Figure 5).

Trade-offs among microbes in nature

As discussed in the 'Introduction' above, it is often difficult to demonstrate the presence of an ecological trade-off in field studies. Thus, it comes as no surprise that there is no direct evidence that phage resistance incurs a competitive cost to bacteria in natural communities. However, several studies provide evidence for the coexistence of phage, phage-resistant and phage-sensitive bacteria in nature, and suggest that coexistence may be due to a trade-off between resistance and competitive ability. For example, Waterbury & Valois (1993) observed that most newly isolated *Synechococcus* strains from marine samples were generally resistant to all co-occurring phage. Phage were present in the samples presumably because they were able to exploit a minority population of phage-sensitive bacteria and thus persist despite the presence of resistant bacteria. This pattern is not unique to aquatic systems; Tan & Reaney (1976) also observed that most *Bacillus* isolates from soil were resistant to all co-occurring phage, and that a minority population of sensitive cells allowed phage to persist. Similarly, Hantula et al. (1991) observed that most bacteria isolated from activated sludge were resistant to co-occurring phage.

Despite these observations, the coexistence of phage, resistant bacteria and sensitive bacteria may not be universal. Estimates of phage production in some marine environments suggest that most if not all bacteria in these environments are sensitive to phage attack (Fuhrman 1999; Wommack & Colwell 2000). It is unclear why phage-resistant bacteria do not dominate in these systems. Possible explanations for these observations include a large competitive cost associated with resistance (a cost too great to allow invasion by resistant types in the oligotrophic open ocean), an advantage to sensitivity due to the influx of nutrients into bacteria cells due to abortive phage infections, and the community-level advantages of maintaining sensitivity (due to nutrient recycling, for example) (Fuhrman 1999; Wommack & Colwell 2000). It is also possible that particle surfaces in the ocean (e.g., on marine snow) may provide spatial refuges for sensitive bacteria, allowing them to exclude resistant mutants.

However, these hypotheses have not yet been tested in field systems.

Conclusions and future directions

Most theories describing the coexistence of species assume that ecological trade-offs play a major role. Using microbial model systems, we have demonstrated that a trade-off between resistance to predators and competitive ability can promote the coexistence of different types of microorganisms, and that the magnitude of such a trade-off can have predictable effects on community structure, dynamics and response to environmental change. The magnitude of such a trade-off is determined by genetic and environmental factors, and thus represents one avenue by which environmental change can alter community properties.

We are currently building on these initial studies, by studying this trade-off in more depth, in different environments and in much more complex communities. These ongoing studies are described below.

The shape of trade-offs

As discussed above, the magnitude of the trade-off between resistance and competitive ability is predicted to be important in determining whether resistance will evolve and persist. However, recent theory suggests that not only the magnitude but also the shape of this trade-off is a crucial factor in determining the outcome of resistance invasion (Boots & Haraguchi 1999). Understanding whether the relationship between resistance and competitive ability is linear, concave, convex, sigmoidal, or some other shape allows specific predictions to be made concerning the most likely relationship between the magnitude of resistance and competitive ability in a given environment, as well as predictions of the effect of altering model parameters on this strategy.

However, this approach has not yet been applied experimentally because the shape of this trade-off has not been estimated for any organism. In future work, we will determine if a relationship exists between the degree of phage resistance and competitive ability in *E. coli*. If a relationship is observed, this relationship will be used to predict the evolutionary and ecological dynamics of laboratory cultures of phage and *E. coli*.

Trade-offs in spatially-structured populations

Our discussion so far has focused on communities in environments with relatively little spatial structure, i.e., where interactions do not vary significantly with space, as in well-mixed batch or chemostat cultures. This is a good starting point for exploring the role of trade-offs in ecological communities; in fact, most theory in ecology and population genetics rests on the assumption of a well-mixed environment. However, this assumption is made primarily for mathematical tractability, and in reality most communities exist in spatially-structured environments, where interactions between individuals are primarily local and the strength of such interactions may diminish with distance. Over the past few decades, there has been an increasing effort to recognize the role of spatial structure in ecological and evolutionary dynamics (Antonovics & Ellstrand 1983; Tilman et al. 1997; Dieckmann et al. 2000) and much effort has been dedicated to the development of new mathematical approaches designed to handle spatial issues (Tilman et al. 1997; Dieckmann et al. 2000).

One reason for the interest in spatial modeling is due to the fact that quite often there are qualitative differences between the results of mean-field (i.e., well-mixed) and spatially explicit models. One dramatic difference is that the range of trade-offs that will allow coexistence of different types is predicted to be different in spatially structured environments than in well-mixed environments. Microbial model systems have been recognized, both theoretically and empirically, as ideal systems with which to explore spatial dynamics (Chao & Levin 1981; Durrett & Levin 1997; Iwasa et al. 1998). We have begun using microcosms of *E. coli* strains in mixed and non-mixed environments to test the predictions of spatially explicit models of communities with ecological trade-offs (Kerr et al. 2002).

Trade-offs and microbial diversity

Simple mathematical models that assume a trade-off between predator resistance and competitive ability can be increased in complexity to model communities with multiple prey, predator or resource types. Such models make predictions not only of population abundance, stability and coexistence but also predict distinct patterns in diversity. For example, Leibold (1996) expanded a simple food chain model to include multiple prey types. This model assumed a trade-off

between prey resistance to the predator and competitive ability. The model predicted a hump-shaped relationship between prey diversity and available energy (i.e., resource input); organisms that were better competitors dominated at lower resource input levels, more resistant types dominated at higher resource input levels, and multiple types coexisted at intermediate input levels. This model has been validated in laboratory studies of bacteria and phage (Bohannan & Lenski 2000a). We are testing this model in more complex communities by estimating bacterial diversity along gradients of environmental energy in aquatic mesocosms. A preliminary study of these mesocosms (Devine et al. unpubl.) found evidence for a hump-shaped relationship between the diversity of some bacterial groups and available energy. However further work is necessary to confirm these preliminary observations.

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