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LETTER

The shape of an ecological trade-off varies with environment

Christine M. Jessup,[1]* and Brendan J. M. Bohannan[2]
[1]Department of Biological Sciences, Stanford University, Stanford, CA, USA 94305
[2]Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, OR, USA 97403
**Correspondence and present address: Division of International Epidemiology and Population Studies, Fogarty International Center, National Institutes of Health, Bethesda, MD, USA 20892. E-mail: cjessup@stanfordalumni.org*

Abstract

Central to most theories that explain the diversity of life is the concept that organisms face trade-offs. Theoretical work has shown that the precise shape of a trade-off relationship affects evolutionary predictions. One common trade-off is that between competitive ability and resistance to predators, parasitoids, pathogens or herbivores. We used a microbial experimental system to elucidate the shape of the relationship between parasitoid resistance and competitive ability. For each of 86 bacteriophage-resistant isolates of the bacterium *Escherichia coli* B, we measured the degree of resistance to bacteriophage T2 (a viral parasitoid) and relative competitive ability in both the resource environment in which strains were isolated and in two alternate environments. We observed that environmental change can alter trade-off shape, and that different physiological mechanisms can lead to different trade-off shapes and different sensitivities to environmental change. These results highlight the important interaction between environment and trade-off shape in affecting ecological and evolutionary dynamics.

Keywords

Adaptive dynamics, bacteria, bacteriophage, divergent selection, *Escherichia coli*, microbial ecology, parasitoid–host, pathogen–host, predator–prey, trade-off.

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INTRODUCTION

Central to most theories about the diversity of life on Earth is the concept that organisms face trade-offs in their ability to allocate limited energy and resources to growth, reproduction and defence (Tilman 2000). Due to physical and chemical constraints, resource allocation limitations and genetic mechanisms such as antagonistic pleiotropy and linkage disequilibrium, traits that confer an advantage for one function may simultaneously reduce the ability to perform other functions. Because traits directly relevant to an organism's fitness are often constrained by other life-history traits (Stearns 1989; Roff 2002), trade-offs form the basis for adaptive radiation and speciation (Dieckmann & Doebeli 1999; Schluter 2000). The role of trade-offs in predator-mediated coexistence has received much theoretical consideration (Leibold 1989, 1996; Grover 1994, 1995). Several empirical ecological studies have also demonstrated the effects of trade-offs on community structure and the maintenance of diversity (e.g. Paine 1966; Lubchenco 1978).

One of the best studied trade-offs is that between competitive ability and resistance to predators, parasitoids, pathogens or herbivores. Resources allocated to anti-herbivore compounds or energy invested in predator avoidance may limit an organism's competitive ability and have important ecological and evolutionary implications. In fact, the observed variation in resistance and immunity among organisms and even within species is assumed to be the result of the fitness costs associated with resistance and defence. Costs of resistance have been investigated in a diverse range of biological systems including insect–parasitoid (e.g. Kraaijeveld & Godfray 1997; Kraaijeveld *et al.* 2001), plant–pathogen (e.g. Bergelson 1994; Biere & Antonovics 1996; Tian *et al.* 2003) and virus–bacteria interactions (e.g. Chao *et al.* 1977; Bohannan & Lenski 1999; Bohannan *et al.* 1999; Brockhurst *et al.* 2004, 2005; Lennon *et al.* 2007). For example, *Drosophila melanogaster* lines that had been artificially selected for increased resistance to a parasitoid wasp were poorer resource competitors (Kraaijeveld & Godfray 1997). Expression of a plant resistance gene, a component of the suite of resistance

genes (R-genes) that act as immune system in plants, was shown to confer a significant reduction in fitness measured in terms of seed production (Tian *et al.* 2003).

Theoretical work has shown that the precise shape of a trade-off relationship can govern ecological interactions and evolutionary dynamics. The importance of the shapes of trade-offs dates back to the work of Richard Levins (Levins 1962, 1968) and has been recently emphasized theoretically in a frequency-dependent context (Boots & Haraguchi 1999; Bowers & White 2002; Jones & Ellner 2004; de Mazancourt & Dieckmann 2004; White & Bowers 2005; Hoyle *et al.* 2008). The use of graphical methods, which superimpose a trade-off relationship on a fitness landscape, has enabled the prediction of evolutionary dynamics with relatively simple geometric methods. From such plots, one can determine which trade-off shapes yield evolutionary branching and which yield evolutionary attractors at intermediate trait values (Hoyle *et al.* 2008). For example, when the relationship is an accelerating cost function (Fig. 1a), models predict that a single evolutionarily stable strategy of intermediate resistance may exist; in contrast, decelerating cost functions yield dimorphism of extreme types.

Even though the predictions of models can depend on trade-off shape, many ecological and evolutionary models ignore trade-off shape, and others assume linear trade-offs. While linear relationships, where the benefits of one trait (e.g. resistance) increase at the same rate as costs of another trait (e.g. competitive fitness), may describe some trade-off functions, the biology underlying trade-offs is also likely to yield nonlinear relationships (Boots & Haraguchi 1999). For example, if resistance increases with the thickness of some passive barrier (e.g. cuticle, gut wall), but the costs of producing a thicker barrier scale with producing the entire volume, resistance becomes increasingly costly and an accelerating cost function results. The costs of resistance may also decrease slower than the benefits, resulting in a decelerating cost function. A possible biological mechanism leading to such a function is discussed in detail by Mealor & Boots (2006).

Trade-offs can be difficult to observe and measure in natural environments – often large sample sizes are required to detect costs, trade-offs may be subtle or absent due to coevolution and trade-offs can vary in different environments. While some studies have demonstrated trade-offs between predator resistance and competitive ability, and one recent study identified negative correlations between the degree of resistance and measures of fitness in an insect host–parasitoid interaction (Gwynn *et al.* 1995), many other studies reveal that fitness costs are absent or undetectable (Simms & Rausher 1987; Bergelson & Purrington 1996; Koricheva 2002). Given the difficulty of measuring trade-offs, elucidating the shape of a trade-off relationship has been particularly challenging. The shape of a trade-off relationship has been inferred experimentally (Mealor & Boots 2006), but there are no reports of direct measurement of trade-off shape.

Trade-offs are also modulated by environmental context. Because few organisms exist in constant environments, it is important to consider how environmental change affects trade-off shape. For example, costs of increasing the concentrations of anti-herbivore chemicals in plant leaves may scale linearly with resistance in one environment. In another environment where resources are limited, resistance may become increasingly costly. The effect of environment on trade-off functions has been explored theoretically (Geritz *et al.* 1998; Jones & Ellner 2004). Empirical work has shown that environment can affect the magnitude of a trade-off (Coley 1986; Bohannan *et al.* 2002; Koricheva 2002), but its effect on trade-off shape is unknown.

Microbial experimental systems are well suited for directly exploring trade-offs. The interactions between bacteriophage and their bacterial hosts offer a predator–prey model (or more precisely, a parasitoid–host system). Bacterial resistance to bacteriophage is usually based on loss or modifications of receptor molecules in the outer membrane. Because these receptors are often important for other cellular functions such as resource uptake and cell wall stability, their loss or modification can have important effects on bacterial growth (Lenski 1988). Research with microbial systems has revealed the effect of resistance trade-offs on bacterial diversification and phage–host coevolution in the laboratory (Chao *et al.* 1977; Bohannan & Lenski 1997, 1999; Brockhurst *et al.* 2004, 2005). Recently a trade-off between virus resistance and competitive ability was also identified and quantified in several isolates of the marine cyanobacterium *Synnechoccus* (Lennon *et al.* 2007). Thus, microbial experimental systems provide not only a powerful platform to investigate theory regarding predator–prey and parasitoid–host interactions, they also provide important insights about the generation and maintenance of microbial diversity in nature.

Microbial systems have also revealed the effect of environment on trade-offs – what is costly in one environment may not be costly in another. For *Escherichia coli*, the magnitude of the trade-off between competitive ability and resistance to phage T4 is different when glucose is the limiting resource than when trehalose is the limiting resource (Bohannan *et al.* 1999). Resistance to phage T4 is also more costly in continuous culture than in batch culture, suggesting that life-history traits important in continuous culture are more affected by mutations conferring phage resistance than those traits important in batch culture (Bohannan *et al.* 2002). Using microbial model systems, one can directly measure trade-offs, determine the effects of genotype and environment on trade-off magnitude, and explore the consequences for community structure (Bohannan *et al.* 2002; Jessup *et al.* 2004, 2005).

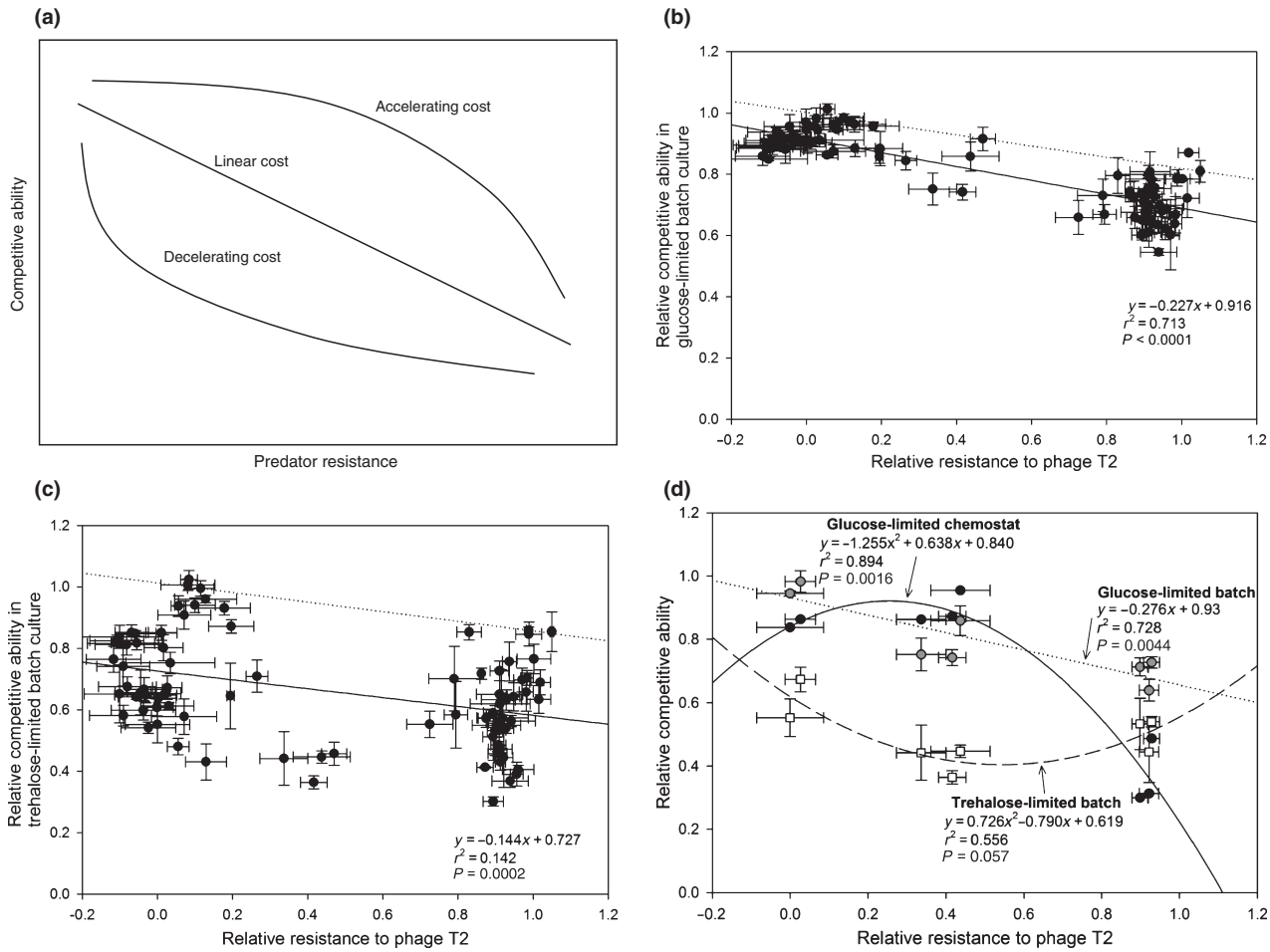

Figure 1 Trade-off shapes in different environments. (a) Conceptual diagram of different trade-off shapes. These shapes yield different evolutionary predictions (see text for details). (b) The trade-off between phage T2 resistance and competitive ability in glucose-limited batch culture. Each value is the mean of several (on average, 5) independent measurements of T2 resistance and several (on average, 4) independent measurements of competitive ability in glucose-limited batch culture for a single strain isolated in this study. Error bars represent standard errors. The negative correlation between resistance and competitive ability was significant. The solid line represents the linear regression of the mean as modelled with the equation presented; the dotted line represented the linear quantile regression of the 95th quantile. (c) The trade-off between phage T2 resistance and competitive ability in trehalose-limited batch culture for the same isolates presented in (b). Each value of competitive ability is the mean of several (on average, 3) independent measurements of competitive ability in trehalose-limited batch culture for each strain isolated. The negative correlation between resistance and competitive ability was significant and the relationship is best described by a linear model. The solid line represents the linear regression of the mean as modelled with the equation presented; the dotted line represents the linear quantile regression of the 95th quantile. (d) The trade-off between phage T2 resistance and competitive ability in glucose-limited continuous culture (black circles) and for the same isolates measured in glucose-limited batch culture (grey circles) and trehalose-limited batch culture (open squares). Each point representing competitive ability in glucose-limited chemostat culture is a single measure. The negative correlation between resistance and competitive ability is significant. In glucose-limited chemostat culture, the relationship is best described by a quadratic model (solid line). For comparison, the same strains measured in glucose-limited batch culture are also presented, and that relationship is linear [as was observed when all strains in glucose-limited batch culture were evaluated (b)] and is represented by the dotted line. The relationship between these strains measured in trehalose-limited batch culture is U-shaped and the quadratic model is represented by the dashed line. Details of statistical tests and results are provided in the text.

In the study detailed below, we used the interaction between phage T2 and *E. coli* B to directly determine the shape of the relationship between parasitoid resistance and competitive ability, and to investigate the effect of environmental change on this shape. We then decomposed the trade-off relationship into components contributed by different physiological mechanisms to examine the mechanisms underlying the observed relationships.

METHODS

Strains of bacteria and bacteriophage

The experiments focus on the interaction between the virulent bacteriophage T2 (ATCC) and *E. coli* B strains REL606 and REL607 (Lenski *et al.* 1991). The strain REL607 is equivalent to REL606 with the exception that it is capable of using the sugar arabinose, a neutral marker in glucose-limited (Lenski 1988; Lenski *et al.* 1991) and trehalose-limited (Travisano & Lenski 1996) environments, enabling assays of competitive fitness. Adsorption of T2 to *E. coli* B has been shown to require two receptors: lipopolysaccharide (LPS) molecules that cover the outer membrane of *E. coli* cells, and outer membrane protein F (OmpF), a porin used for the uptake of glucose (Hantke 1978; Lenski 1984). Other phages use each of these receptors exclusively: Tu1a attaches to OmpF (Hancock & Reeves 1975; Datta *et al.* 1977), and T4 and T7 both require the LPS core (Prehm *et al.* 1976; Wright *et al.* 1980), but the binding site for T7 is deeper in the LPS core than is the binding site for T4 (Lenski 1988). Mutations that affect the LPS molecule can also indirectly affect assembly of outer membrane proteins such as OmpF and LamB (Laird *et al.* 1994). Because several phages use receptors that overlap with the receptor requirements of T2, selection of *E. coli* resistant to each of these phages can yield strains that exhibit the correlated trait of partial resistance to T2.

Pre-existing, spontaneously occurring, phage-resistant isolates were independently selected using standard techniques (Lenski 1988) with each of the following phages: Tu1a, T4, T7, T2. Resistance to Tu1a, T4 and T7 has been shown to confer partial to complete cross-resistance to T2, and therefore selecting with these strains provides a means to obtain isolates with a range of T2-resistance levels (in contrast, selection in the presence of T2 generally yields complete T2 resistance). Approximately 10^8 cells from independently inoculated overnight cultures of the ancestral strain, grown in Davis minimal glucose (MG) broth supplemented with 1000 μg glucose mL^{-1} and 2×10^{-3} μg thiamine hydrochloride mL^{-1} (DM1000-glu) were mixed with concentrated phage lysate in a Davis MG agar overlay on Davis MG plates and incubated overnight. This procedure of inoculating multiple initial cultures with cells from an archival stock of the phage-sensitive ancestor (−80 °C) and using each of these cultures to obtain one phage-resistant isolate ensures that each resistance-conferring mutation arises independently (although it does not preclude the possibility that multiple isolates harbour the same mutation). In this way, we obtained a number of independently derived strains of bacteria that exhibit resistance to the phage used in selection. For each selecting phage, we selected at least 20 phage-resistant isolates (i.e. 20 T4-resistant isolates, 20 T7-resistant isolates, 20 T2-resistant isolates); for Tu1a, we originally selected 20 resistant isolates but upon learning that these Tu1a-resistant isolates exhibited intermediate resistance levels, we generated 40 additional isolates in an effort to fill in this region of trade-off space. As described below, not all of these isolates were included in the analysis. Resistant colonies were purified by sequential streaking in order to remove residual phage, then the isolates grown to mid-log phase in DM1000-glu, at which time their phage-resistance profiles were determined by streak assay against T2, T4, Tu1a, T7, lambda, T5 and T6, and stored in 20% glycerol solution at −80 °C for further analysis. The isolates obtained by this method are likely to harbour single mutations, as the probability of double mutants arising is extremely small (Lenski & Levin 1985).

Phage T2 resistance

Resistance to phage T2 was measured by calculating the proportion of cells surviving incubation with phage T2. Isolates were inoculated from −80 °C stocks into DM1000-glu and grown overnight. Cultures were diluted into Davis minimal broth supplemented with 25 μg glucose mL^{-1} and 2×10^{-3} μg thiamine mL^{-1} (DM25-glu) and grown overnight. Cultures were then diluted 1 : 100 into fresh DM25-glu and grown overnight to stationary phase. The culture was sampled to enumerate the starting bacterial density and then inoculated with T2 at a multiplicity of infection (the ratio of phage to bacteria) of *c.* 8–10. After 1 h of incubation at 37 °C, surviving cells were enumerated by diluting and plating on MG plates. Assays were conducted in blocks of up to 40 isolates. Within each block of assays, four independent replicates of the phage-sensitive parental strain, REL606 or REL607, were included to determine the ancestral sensitivity to T2 as measured by this assay.

For each isolate, the death rate was calculated as the $\log_e$ of the ratio of the concentration of bacteria at the start of the assay to the concentration following incubation with phage, yielding a measure of phage sensitivity. Because cells were in stationary phase, resources were exhausted and no bacterial growth occurred during the assay. Relative resistance to T2 was computed as one minus the ratio of sensitivity of the test strain to the mean of four replicate measures of sensitivity for the ancestral strain assayed in the same block using the following equation:

$$\text{Resistance} = 1 - \frac{\ln\left(R_0/R_{60}\right)}{\ln\left(N_0/N_{60}\right)}, \qquad (1)$$

where R_0 and R_{60} are the densities of the test strain at the beginning and end of the assay and N_0 and N_{60} are the densities of the phage sensitive strain at the beginning and end of the assay, respectively. Assays were replicated an average of five times for each phage-resistant isolate.

All assays of resistance were conducted under glucose-limited conditions, and therefore the *x*-axes of the presented trade-off plots are identical. Although some outer membrane proteins, and thus phage receptors, respond to environmental change (e.g. OmpF and OmpC abundance in outer membrane changes in response to osmolarity in *E. coli* K12), OmpF is constitutively expressed in *E. coli* B (Pugsley & Rosenbusch 1983; Schneider *et al.* 2002). Thus, for a given isolate, the amount of OmpF in the outer membrane is not expected to vary with environment. Furthermore, for the ancestral strain REL607, we measured resistance to T2 in both DM25-tre and DM25-glu and observed no effect of environment (data not shown).

The selection procedures yielded some isolates that exhibited increased sensitivity to T2. Because the goal of this work was to elucidate costs of resistance, isolates exhibiting increased sensitivity were excluded from further analyses. Isolates were included if the 95% confidence interval of resistance either overlapped zero or was greater than zero.

Competitive ability

The competitive cost associated with different levels of T2 resistance was determined through direct competition with the reciprocally marked T2-sensitive ancestor in the absence of T2 for all isolates in the selected environment, glucose-limited batch culture, and in two alternate environments: trehalose-limited batch culture and for a subset of isolates, glucose-limited continuous culture (chemostats). These alternate environments were chosen because absolute trade-offs have been shown to differ in magnitude in these environments (Bohannan *et al.* 2002). Batch competition assays were conducted in Davis minimal media supplemented with 2×10^{-3} μg mL^{-1} thiamine hydrochloride and either 25 μg glucose mL^{-1} or 25 μg trehalose mL^{-1} using standard techniques (Lenski *et al.* 1991). Competing strains were co-inoculated fresh DM25-glu or DM25-tre. A sample of the competition culture was serially diluted and plated on tetrazolium agar (TA), which enables the competitors to be differentiated, at the start of the assay and following 24 h of incubation at 37 °C. Relative competitive ability was calculated as:

$$W_{b_{R,S}} = \frac{m_R}{m_S} = \frac{\ln(R_{24}/R_0)}{\ln(N_{24}/N_0)}, \quad (2)$$

where m_R and m_S are the Malthusian parameters of the resistant strain and sensitive ancestor respectively, calculated as the number of doublings in 24 h. Batch competition assays were replicated an average of four times for each phage-resistant genotype in glucose-limited batch culture and three times in trehalose-limited batch culture.

Because costs of complete phage resistance have been shown to differ in batch culture and continuous culture, relative competitive ability was measured for a subset of strains under continuous culture conditions through pair-wise competition against the reciprocally marked phage-sensitive ancestor (REL606) in glucose-limited (DM25-glu) chemostats maintained at 37 °C at a dilution rate of 0.2 turnovers h^{-1}. These continuous culture vessels are similar to those described in Chao *et al.* (1977). Competing strains were co-inoculated into chemostats, which were then sampled two to three times daily. At each sampling, phage-sensitive and phage-resistant populations were enumerated by dilution and plating on TA plates. Relative competitive ability in the chemostat environment was calculated as described in Lunzer *et al.* (2002) using the equation:

$$W_{c_{R,S}} = \frac{\mu_R}{\mu_S} \approx \frac{1 + qs/D}{1 - ps/D}, \quad (3)$$

where μ_R and μ_S are the growth rates of the phage-resistant and phage-sensitive strains respectively, D is the chemostat dilution rate, p and q are the mean relative frequencies of the resistant and sensitive strains respectively over the time period analyzed and s is the selection coefficient that was calculated as the slope of the $\log_e$ ratio of resistant and sensitive densities against time by linear regression. All competition assays were conducted in a single block with all chemostats operating under the same conditions and receiving media from the same reservoir. One chemostat competition assay was conducted per isolate.

Phenotypic characterization

Isolates were grouped into phenotypic classes based on their sensitivity to the hydrophobic antibiotic novobiocin and their profiles of cross-resistance to other phages with known receptor usage. Mutations that cause defects in the LPS core cause cells to be more sensitive to hydrophobic compounds such as the novobiocin (Lenski 1988). Strains were characterized as novobiocin-resistant if overnight cultures of isolates in DM1000-glu supplemented with 125 μg mL^{-1} novobiocin grew to the same visual turbidity as wild-type strains REL606 and REL607. All strains, including the ancestral novobiocin-resistant strain, were inoculated into DM1000-glu from −80 °C stocks and grown overnight, then diluted into 1-mL fresh medium containing 125 μg mL^{-1} novobiocin and 1-mL fresh medium without antibiotic and incubated overnight at 37 °C. Cultures that exhibited similar turbidity in DM1000-glu and in novobiocin-supplemented DM1000-glu were categorized as novobiocin-resistant. Cultures that exhibited a reduction in turbidity in the novobiocin-supplemented medium

compared to DM1000-glu were categorized as novobiocin-sensitive.

Phage-resistance profiles were determined by streak assay. Overnight DM1000-glu cultures of each of the isolates were spread across a line of concentrated phage lysate on MG plates. The following phages were tested: T2, T4, T7, Tu1a and lambda. Strains were categorized as resistant to a particular phage if growth was not inhibited by the phage lysate. Strains exhibiting reduced growth in the presence of phage lysate were classified as partially resistant.

Combining the novobiocin and phage-resistance data, isolates were grouped into three broad mutational classes: LPS-1, LPS-2 and LPS-3. These classes reflect different classes of the LPS mutations. LPS-1 mutants exhibited resistance to the LPS-specific phages T4 and T7, but maintained novobiocin resistance. LPS-2 mutants exhibited resistance to the OmpF-specific phage Tu1a and sensitivity to novobiocin. The LPS-3 group contained mutants that appeared to exhibit severely truncated LPS molecules, as these isolates were resistant to all phages tested and sensitive to novobiocin.

Data analysis

Variation in resistance and competitive ability in each environment was described by one-way ANOVA. A trade-off between resistance and competitive ability in each environment was determined by a negative correlation between these two traits. The shape of the trade-off was determined using ordinary least-squares linear and polynomial regression and using quantile regression. Although many models assuming particular trade-off shapes assume nonlinear functions (Boots & Haraguchi 1999; Jones & Ellner 2004), we employ linear regression using nested polynomial models in order to directly compare nested model fits. Model fits were compared using a partial F-test. While least-squares regression determines the best fit line that minimizes squared residual errors around the mean of the dependent variable, quantile regression finds the best fit line that minimizes the sum of absolute values of residual errors around a specified quantile of the dependent variable (Knight & Ackerly 2002; Cade & Noon 2003). The 95th quantile regression identifies the best fit line where 95% of observations fall below this line and 5% of observations fall above it. For trade-offs, it may be more evolutionarily relevant to consider an upper quantile (e.g. 95th), as this reflects the upper boundary of competitive ability.

Comparisons of mutational classes in different environments were made using Welch's approximate t-test, and directional comparisons were made using the one-tailed test. ANOVAs, linear and quadratic regressions were determined using JMP 4.0 statistical software (SAS Institute, Inc. Cary, NC, USA) and R-statistical analysis software. Quantile regression analysis was conducted using the 'quantreg' library (Koenker 2006) of R-statistical analysis software (R2.2.1, 2005, distributed freely at http://www.R-project.org/).

RESULTS

Variation in phage-resistance and competitive ability in *E. coli*

Of the 86 strains isolated by selection with T2, T4, T7 or Tu1a, 53 isolates exhibited significant resistance to phage T2 (i.e. the 95% confidence interval did not overlap zero) and 33 isolates exhibited resistance levels that were not different from the T2-sensitive ancestor (the 95% confidence interval overlapped zero). Isolates exhibited significant variation in measured T2 resistance (one-way ANOVA: $F_{85,357} = 58.96$, $P < 0.0001$), competitive ability in glucose-limited batch culture (one-way ANOVA: $F_{85,317} = 12.76$, $P < 0.0001$) and competitive ability in trehalose-limited batch culture (one-way ANOVA: $F_{85,202} = 10.62$, $P < 0.0001$). Variation in competitive ability was even observed among strains with mutations that did not confer T2 resistance (i.e. relative resistance not different from zero). This is not surprising given the selection techniques. For example, strains with mutations conferring resistance to the selecting phage Tu1a may incur a cost, but some of these mutations may have no effect on resistance to T2.

The shape of the trade-off

Phage T2 resistance and competitive ability in glucose-limited batch culture exhibited a significant negative

Table 1 The relationship between T2 resistance and competitive ability in each of the environments tested

Competition environment	Pearson correlation coefficient	Significance
Glucose batch	−0.8463	$P < 0.0001$
LPS-1	0.3485	ns
LPS-2	−0.5762	$P = 0.0026$
LPS-3	−0.1941	ns
Trehalose batch	−0.3901	$P = 0.0002$
LPS-1	0.3901	ns
LPS-2	−0.6058	$P = 0.0013$
LPS-3	0.2485	ns
Glucose chemostat	−0.8426	$P = 0.0086$
LPS-1	–	–
LPS-2	0.6644	ns
LPS-3	0.7193	ns

Significance values are provided and correlations that are not significant at $P < 0.05$ are indicated by 'ns'.

correlation (Table 1). The relationship was better fit by the linear regression model ($r^2 = 0.713$, $P < 0.0001$) than a quadratic model ($F_{1,83} = 0.353$, $P = 0.554$) (Fig. 1b, Table 2). The best-fit quantile regression of the 95th quantile was also linear (Table 2) and fell close to the fit of the mean (Fig. 1b).

In both trehalose-limited batch culture and glucose-limited chemostat culture, phage T2 resistance and competitive ability also exhibited significant negative correlations (Table 1). In trehalose-limited batch culture, there was much more variability in competitive ability, reflected in both increased measurement error and increased variance among strains. The trade-off relationship in trehalose-limited batch culture was best described by a linear regression model ($r^2 = 0.142$, $P = 0.0002$; $F_{1,83} = 2.49$, $P = 0.118$; Fig. 1c, Table 2). Although the relationship looks curvilinear, neither the linear nor the quadratic model explained much of the variation. The best fit of the 95th quantile of the data was also a linear model (Table 2). The difference between the fits of the 95th quantile and the mean is much larger in

Table 2 The overall effect of phage T2 resistance on competitive ability in each of the environments tested: glucose-limited batch culture, trehalose-limited batch culture and glucose-limited chemostat culture

Environment	Model	Term	Coeff.	SE coeff.	t	P	r^2	d.f.
Glucose	Mean	Overall model				< 0.0001	0.713	1
	Linear	Constant	0.916	0.010	91.04	< 0.0001		
		Resist	−0.227	0.016	−14.56	< 0.0001		
	Mean	Overall model				< 0.0001	0.711	2
	Quadratic	Constant	0.915	0.010	90.28	< 0.0001		
		Resist	−0.178	0.083	−2.15	0.0344		
		Resist^2	−0.053	0.088	−0.594	0.5540		
	95th Quantile	Constant	1.00	0.012	86.67	< 0.0001		1
	Linear	Resist	−0.183	0.031	−5.83	< 0.0001		
	95th Quantile	Constant	0.989	0.019	51.83	< 0.0001		2
	Quadratic	Resist	−0.145	0.070	−2.080	0.041		
		Resist^2	−0.025	0.073	−0.340	0.734		
Trehalose	Mean	Overall model				0.0002	0.142	1
	Linear	Constant	0.727	0.024	30.25	< 0.0001		
		Resist	−0.144	0.037	−3.88	0.0002		
	Mean	Overall model				0.0003	0.153	2
	Quadratic	Constant	0.730	0.024	30.54	< 0.0001		
		Resist	−0.447	0.195	−2.29	0.0247		
		Resist^2	0.329	0.208	1.58	0.118		
	95th Quantile	Constant	1.013	0.029	34.21	< 0.0001		1
	Linear	Resist	−0.156	0.035	−4.50	< 0.0001		
	95th Quantile	Constant	1.038	0.045	23.04	< 0.0001		2
	Quadratic	Resist	−0.404	0.274	−1.47	0.144		
		Resist^2	0.218	0.268	0.816	0.417		
Chemostat	Mean	Overall model				0.0086	0.662	1
	Linear	Constant	0.983	0.096	10.28	< 0.0001		
		Resist	−0.598	0.156	−3.83	0.0086		
	Mean	Overall model				0.0016	0.894	2
	Quadratic	Constant	0.840	0.066	12.79	< 0.0001		
		Resist	0.638	0.341	1.87	0.1203		
		Resist^2	−1.255	0.334	−3.75	0.0133		

Fits of the linear and quadratic regression models of the mean and of the 95th quantile of competitive ability against resistance are presented. Glucose, glucose-limited batch culture; Trehalose, trehalose-limited batch culture; Resist, phage T2 resistance; Coeff., regression coefficient for each term; SE coeff., standard error of the coefficient; t, value of the t-test; P, probability value; r^2, adjusted R-squared; d.f., degrees of freedom for overall model.

trehalose-limited batch culture than in glucose-limited batch culture, reflecting the larger variance.

In glucose-limited chemostat culture, resistance and competitive ability also exhibited a significant negative correlation, and the relationship between resistance and competitive ability was best described by quadratic regression ($r^2 = 0.894$, $P = 0.0016$; quadratic term: $P < 0.05$; $F_{1,5} = 14.09$, $P = 0.0133$; Fig. 1d, Table 2). The relationship was hump-shaped. For comparison, the same strains measured in glucose-limited batch culture exhibited a linear relationship ($r^2 = 0.728$, $P = 0.0044$), as was observed when all strains in glucose-limited batch culture were evaluated (Fig. 1b). Although competitive ability in chemostat culture was measured only once for each isolate, it should be noted that at both low- and high-resistance levels, these values fall below the 95% confidence interval of the mean competitive ability of the same strain in glucose-limited batch culture; of the three isolates exhibiting intermediate resistance, chemostat competitive ability for two of these isolates falls above the upper limit of the 95% confidence interval of the same isolate's mean competitive ability in batch culture (Fig. 1d).

The relationship between resistance and competitive ability for the subset of strains assayed in glucose-limited chemostat culture was also different when these strains where studied in trehalose-limited batch culture (Fig. 1d). The relationship for these strains in trehalose-limited batch culture was best described by a U-shaped quadratic regression model ($r^2 = 0.556$, $P = 0.0567$; quadratic term: $P < 0.05$). This model provided a significantly better fit by the trehalose data than a linear model ($F_{1,5} = 9.32$, $P = 0.0283$). At low- and intermediate-resistance levels, competitive ability in trehalose-limited batch culture was lower than that in glucose-limited batch culture and glucose-limited chemostat culture (95% confidence intervals did not overlap).

Mutational differences underlying the trade-off

The wild-type ancestral strain was resistant to novobiocin and sensitive to all phages tested by the streak assay. Derived isolates were classified into three groups (Table 3) as follows. Strains exhibiting T4 and T7 resistance were classified as LPS-1 mutants because they harbour an alteration in the LPS molecule that affects binding by these LPS-specific phages (Lenski 1988). However, the alteration was not severe enough to affect sensitivity to novobiocin or the assembly of outer membrane proteins that serve as receptors for the other phages tested. Strains that were sensitive to novobiocin were classified as LPS-2 and LPS-3 mutants, and these strains harbour mutations that more severely truncate the LPS molecule. For LPS-2 mutants, this indirectly affects OmpF because strains in this group were resistant to the OmpF-specific phage Tu1a. For these mutants, LamB is also affected because growth, albeit reduced growth, was observed in the presence of bacteriophage lambda (partially resistant). For LPS-3 mutants, the affect on LPS is most severe – these strains were resistant to all phage tested, suggesting a dramatic effect on the assembly of several outer membrane proteins. Although the selection procedure was expected to yield strains with mutations in OmpF or LPS-related genes, all strains in this study appear to harbour mutations in the genes involved in LPS synthesis and these mutations indirectly affect OmpF and LamB by affecting the assembly of these outer membrane proteins (Randall 1975; Laird *et al.* 1994). No strains with mutations in the OmpF gene were observed (as noted above, strains that appeared to be resistant only to the OmpF-specific phage in the phage profiling also exhibited sensitivity to novobiocin, indicating that they were actually LPS mutants.) This may reflect differences in mutational targets: a functional OmpF porin is a trimer encoded by a single and relatively small gene; LPS is a larger and more complex molecule assembled through the action of proteins encoded by several larger genes. Thus, LPS is a much larger target for mutation. Results of this classification yielded 20 LPS-1 strains, 25 LPS-2 strains and 41 LPS-3 strains.

Classifying strains based on these mutational classes revealed clustering in the trade-off between resistance and competitive ability (Fig. 2). Strains that were in the LPS-1 mutational class were marginally resistant to T2 and exhibited little cost of resistance to T2 in any of the tested environments. Competitive ability in strains classified in this group was significantly higher (cost was lower) in glucose-limited batch culture than in trehalose-limited batch culture (Welch's approximate t-test: $t_{27.49} = 2.29$, $P = 0.030$). Within this mutational group, competitive ability was not

Table 3 Classification of strains into mutational classes based on resistance to chemical and biological agents of known target specificity

		Hydrophobic antibiotic sensitivity	Phage cross-resistance profile				
Mutational class	*n*	Novobiocin 125 mg mL^{-1}	T2	T4	T7	Tu1a	lambda
Wild type	–	Resistant	S	S	S	S	S
LPS-1	20	Resistant	S	R	R	S	S
LPS-2	25	Sensitive	S	S	S	R	PR
LPS-3	41	Sensitive	R	R	R	R	R

S, sensitive; R, resistant; PR, partially resistant (reduced growth in the presence of phage).

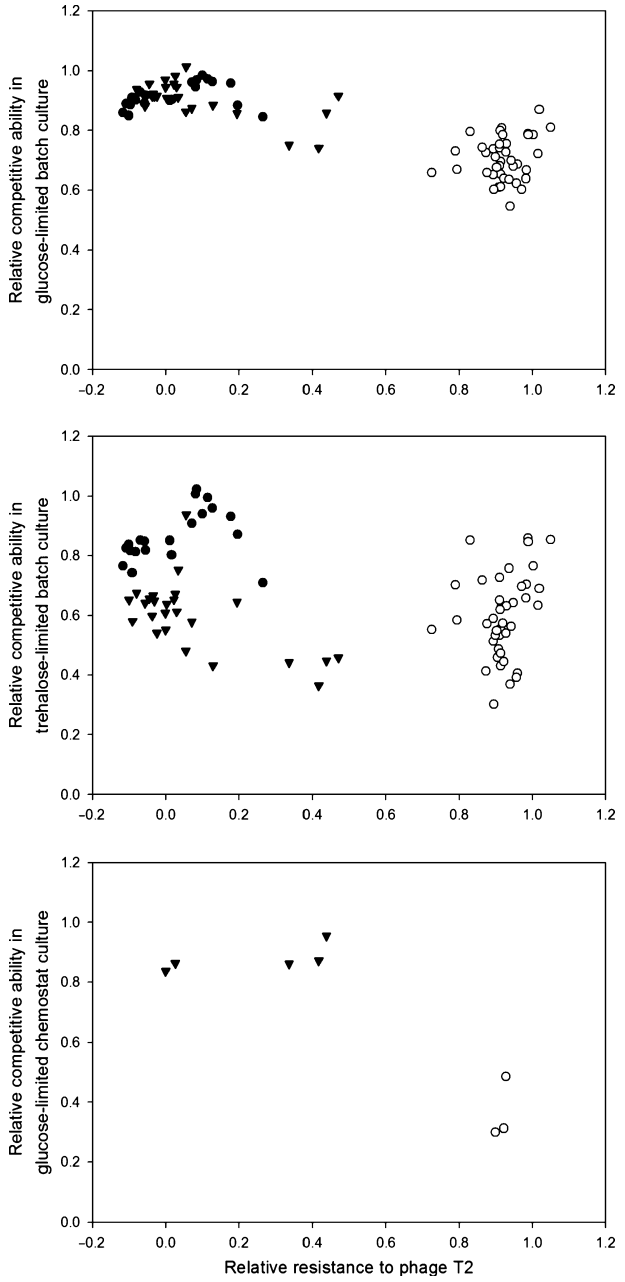

Figure 2 The trade-off between phage T2 resistance and competitive ability in (a) glucose-limited batch culture, (b) trehalose-limited batch culture and (c) glucose-limited chemostat culture with strains labelled by mutational class. Closed circles represent LPS-1 mutations as determined by resistance to novobiocin as described in the text, closed triangles represent LPS-2 mutations and open circles represent LPS-3 mutations in which the LPS molecule is severely truncated, conferring sensitivity to novobiocin and resistance to all phages tested.

correlated with resistance in any environment tested ($r = 0.3485$, $P = 0.1321$ for glucose-limited batch culture and $r = 0.3901$, $P = 0.0891$ for trehalose-limited batch culture; no LPS-1 strains were measured in chemostat culture). Strains in the LPS-3 mutational class exhibited high levels of T2 resistance (often complete resistance) and high costs associated with this resistance. The cost of T2 resistance in this class of mutants was significantly higher in trehalose-limited batch culture than in glucose-limited batch culture (Welch's approximate t-test: $t_{65.81} = 4.80$, $P < 0.0001$) and was significantly higher in glucose-limited chemostat culture than in glucose-limited batch culture (Welch's approximate t-test: $t_{2.20} = 5.71$, $P = 0.0236$). However, competitive ability was not correlated with resistance level in glucose-limited batch culture ($r = 0.194$, $P = 0.224$), trehalose-limited batch culture ($r = 0.249$, $P = 0.117$) or glucose-limited chemostat culture ($r = 0.7193$, $P = 0.489$).

Strains classified as harbouring LPS-2 mutations spanned a range of resistance levels and several exhibited intermediate levels of resistance. Competitive ability in this group is significantly negatively correlated with resistance level in both glucose-limited ($r = -0.576$, $P = 0.0026$) and trehalose-limited batch cultures ($r = -0.606$, $P = 0.0013$). In both glucose-limited and trehalose-limited batch cultures, the relationships between resistance and competitive ability for the LPS-2 group are best fit by linear regression models (Table 4). In this group of strains, competitive ability was significantly lower in trehalose-limited batch culture than in glucose-limited batch culture (Welch's approximate t-test: $t_{35.83} = 11.41$, $P < 0.0001$). However, there was no overall significant difference between competitive ability in glucose-limited batch and glucose-limited chemostat cultures (Welch's approximate two-tailed t-test: $t_{7.39} = 1.12$, $P = 0.298$).

DISCUSSION

Although the predictions of most models that describe species interactions and phenotypic evolution depend on the nature of the relationship between life-history characters, the shape of the relationship remains poorly understood (Boots & Haraguchi 1999; Roff 2002; Boots & Bowers 2004). In this work, the shape of the trade-off was characterized and consequences of environmental change on this relationship were investigated. In each of the environments, different shapes of the trade-off relationship were observed. In the selected resource environment, the cost of resistance to T2 scaled linearly with the degree of resistance. In a different resource environment (trehalose-limited batch culture), isolates of intermediate phage resistance exhibited very low fitness. Although this suggests a change in shape, our nested polynomial regression analyses did not detect this as a significant change in shape when all isolates were considered. In continuous culture, the strains exhibited a hump-shaped relationship. Interestingly, these

Table 4 The effect of phage T2 resistance on competitive ability for isolates in mutational class LPS-2

Environ.	Model	Term	Coeff.	SE coeff.	t	P	r^2	d.f.
Glucose	Mean	Overall model				0.0026	0.303	1
	Linear	Constant	0.919	0.011	82.29	< 0.0001		
		Resist	−0.211	0.062	−3.38	0.0026		
	Mean	Overall model				0.0116	0.273	2
	Quadratic	Constant	0.920	0.012	78.40	< 0.0001		
		Resist	−0.179	0.179	−1.00	0.328		
		Resist^2	−0.086	0.456	−0.189	0.852		
Trehalose	Mean	Overall model				0.0013	0.339	1
	Linear	Constant	0.628	0.0211	29.66	< 0.0001		
		Resist	−0.432	0.118	−3.65	0.0013		
	Mean	Overall model				0.0046	0.331	2
	Quadratic	Constant	0.633	0.0219	28.872	< 0.0001		
		Resist	−0.167	0.335	−0.500	0.622		
		Resist^2	−0.720	0.851	−0.846	0.406		

Fits of the linear and quadratic regression models of the mean competitive ability against resistance are presented. Abbreviations are as in Table 2.

isolates, which were selected because they spanned a range of resistance levels, exhibited a relationship in trehalose-limited batch culture that was best fit by a U-shaped quadratic model.

The observation that the relationship in the selected environment exhibited less variance than the relationship in an alternate resource environment is not surprising. The strain selection process we used essentially selects strains that are both resistant to the selecting phage and grow relatively well in glucose-limited culture. The benefit of this approach is that it captures relevant mutations in a given environment. However, given that strains were not selected for their ability to utilize alternate resources, it is not surprising that strains exhibited greater variability in the cost of resistance in trehalose-limited batch culture at all levels of phage resistance.

While differences in variance can be explained by the selection process, differences in the shape of the relationship reflect more fundamental differences in the mechanisms underlying the trade-off. In glucose-limited chemostat culture, an accelerating cost function was observed (Fig. 1d), with greater costs of high T2 resistance in chemostat culture than in batch culture, and relatively low costs of intermediate resistance. The results build upon earlier observations that the cost of complete resistance is higher in chemostat than in batch culture (Bohannan *et al.* 2002). However, it also appears that mutations conferring intermediate levels of resistance are less costly in chemostat culture than in batch culture. Taken together, these results suggest that mutations conferring high levels of resistance negatively affect the ability to grow at low resource concentration more than they affect the ability to grow when resources are abundant. For mutations conferring intermediate levels of resistance, this appears to be the opposite: these mutations negatively affect the ability to grow when resources are abundant but not when resources are scarce (Fig. 1d).

In trehalose-limited batch culture, a U-shaped relationship between phage resistance and competitive ability was observed for the subset of strains measured in all three environments – strains of intermediate resistance paid the highest cost of resistance. This relationship may seem inconsistent with life-history theory, which defines trade-offs as negative interactions between traits (Stearns 1989). However, the detailed molecular and physiological understanding of these microbial systems provides an explanation. By elucidating the underlying mechanisms of T2 resistance and partial resistance in this system, it becomes clear that different classes of mutants exhibit different trade-offs and together, these trade-offs yield the observed relationships. By screening isolates for resistance to phages with known receptor usage, strains were classified into three groups and these three groups cluster in distinct regions of trade-off space. Interestingly, the relationship in trehalose-limited batch culture is delimited by two mutational clusters that each exhibit no relationship between resistance and competitive ability (LPS-1 and LPS-3), and one group of mutants that exhibits a significant negative linear relationship between resistance and competitive ability (LPS-2). In the glucose-limited batch environment, similar clustering was observed: only one class of mutants (LPS-2) exhibits a negative relationship between resistance and competitive ability and the two other classes exhibit no relationship.

Evaluating this pattern of mutational classes in the chemostat environment is somewhat more difficult due to the limited sample size. However, clustering was again observed. Thus, some classes of mutants exhibit no relationship between resistance and competitive ability and others exhibit negative relationships. Together, these patterns underlie the overall functional form of the trade-off and drive differences in the shape of the trade-off in different environments. The observation of clustering in mutational groups may also be a product of the simplicity of this experimental system. In this system, a single mutation can confer a dramatic change in phage resistance and its associated cost.

Because all the strains analysed in this study appear to harbour mutations resulting in defective LPS molecules, the observation of different relationships in glucose- and trehalose-limited batch culture is likely the result of the indirect effects of LPS mutations on the assembly of OmpF and LamB, the preferred transport channels for glucose and trehalose respectively. Strains that exhibited a dramatic reduction in competitive ability in trehalose compared to glucose (class LPS-2) were resistant to phage Tu1a and appeared to be partially resistant to phage lambda. For such mutants with defective OmpF porins, glucose may still be able to enter the cell through LamB (Klein & Boos 1993). Consequently, mutant strains in a glucose-limited environment may still be fairly competitive compared to the wild-type ancestor. However, the effect of defective LPS on LamB may limit the ability of the cell to uptake trehalose, and so the same LPS mutation in a trehalose-limited environment is much more costly. Additional research on the nature of the LPS mutations and analysis of proteins in the outer membrane may better elucidate the exact mechanisms underlying these trade-offs.

The possibility that some isolates are identical to one another, which may bias our curve-fitting analysis, cannot be excluded. Although we employed experimental methods that maximize the chances of obtaining independent mutations, some isolates may harbour the same mutation and exhibit the same phenotype in terms of resistance and competitive ability. The collection of isolates we studied in these experiments was not a random collection of spontaneous mutants. Obtaining such a pool is possible through random mutagenesis; however, we were interested in obtaining an ecologically and evolutionary relevant collection of isolates. In its natural enteric habitat, *E. coli* interacts with some or all of these phages at different times. Therefore, selecting with these phages provides not only a means to observe a spectrum of T2-resistance levels, but also offers the opportunity to address ecologically relevant selective pressures. Although number of strains in a given phenotypic class or at a given resistance level may influence the precise fit of the regression models, we emphasize that the strains of intermediate resistance exhibit different costs in different environments.

Microbial experimental systems offer the ability to generate large numbers of strains and characterize these strains under controlled laboratory settings. Despite this control, variance around the trade-off relationships was observed, and the extent of this variance differed by environment. That such variation is observed in controlled, simplified laboratory systems underscores the challenges faced by biologists searching for trade-offs in plant and animal populations. That said, the observation that environmental change affects the shape of a trade-off in these microbial systems leads to several testable predictions, particularly regarding the distribution of resistant types in a given community. One of the advantages of the geometric approach for analysing evolutionary dynamics is that the type of evolutionary behaviour predicted depends on the shape of the trade-off and does not require that the trade-off be explicitly specified (Hoyle *et al.* 2008). For example, if the strains isolated in this work were placed in a glucose-limited chemostat environment with phage T2, one would expect persistence of a strain exhibiting intermediate resistance due to the accelerating cost function observed in this environment.

Finally, in order to evaluate this relationship, the focus was limited to one side of this host–parasitoid interaction – the nature of the trade-off for strains that vary in their degree of resistance to T2. However, in most host–parasitoid or predator–prey systems, populations are coevolving. Evolution of partial resistance or complete resistance in the bacterial population may be countered by host-range mutations in the bacteriophage (Lenski & Levin 1985). Resistance to these host-range mutants may set up entirely different cost functions depending on the nature of the evolving interaction between phage and host. Each turn of the coevolutionary wheel may set up new trade-off relationships.

For decades, researchers have sought to understand the vast diversity of anti-herbivore defence mechanisms (Stamp 2003). In microorganisms, the observed extraordinary diversity of bacteria may be, to some extent, the result of trade-offs in the coevolution with bacteriophage (Weinbauer & Rassoulzadegan 2004). Because trade-offs affect ecological interactions and subsequently drive evolutionary change, they fundamentally underlie the evolution of biodiversity. The role of environmental heterogeneity has long been implicated as a driver of ecological diversity (Huston 1994; Rosenzweig 1995). The observation that environmental change affects the shape of an ecological trade-off, and the shape of the trade-off, in turn, affects ecological and evolutionary dynamics suggests an even greater role for environmental heterogeneity in the maintenance of diversity.

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