EVOLUTIONARY TRADE-OFFS UNDER CONDITIONS OF RESOURCE ABUNDANCE AND SCARCITY: EXPERIMENTS WITH BACTERIA

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Abstract. It is often hypothesized that bacteria that are superior competitors when resources are abundant must be inferior competitors when resources are scarce, and vice versa. Most previous studies that sought to test this trade-off hypothesis compared kinetic parameters of extant strains of bacteria, with mixed results. We employed an experimental approach in which bacterial populations were propagated for many generations under two distinct regimes and their evolutionary responses were monitored. Thirty-six populations of bacteria were allowed to adapt evolutionarily to either abundant (batch culture) or scarce (chemostat culture) resource regimes. The competitive fitness of each derived line, relative to its ancestor, was then measured under both regimes. The trade-off hypothesis predicts that adaptation to either selective regime causes a concomitant loss of fitness under the alternative regime. Overall, our findings failed to support this hypothesis, and several cases contradict it. Only two derived lines showed clear trade-offs, having significantly adapted to the selective regime while becoming significantly less fit in the alternative regime. By contrast, five derived lines significantly improved in the alternative regime even as they adapted to their selective regime. Summing over all 36 derived lines (including those for which the observed fitness changes were nonsignificant under one or both regimes), 15 cases support the trade-off hypothesis, whereas 21 indicate the opposite result. These data therefore refute the necessity, or even general tendency, for evolutionary trade-offs in performance under conditions of resource abundance vs. scarcity. Instead, these data suggest that bacteria are able to adapt to a particular level of resource via multiple evolutionary pathways, which may produce either gains or losses in fitness at some different level of resource.

Key words: bacteria; bioremediation; competition; experimental evolution; microbial ecology; r and K selection; resource availability; trade-offs.

INTRODUCTION

Evolutionary trade-offs in performance from one environment to another have long been thought to be important in limiting the distribution and abundance of organisms. These trade-offs are the product of genetic, physiological, and material constraints that prevent an organism from simultaneously optimizing different traits (Stearns 1992). Trade-offs have been hypothesized, and sometimes demonstrated, between such traits as maximum growth rate and carrying capacity (MacArthur and Wilson 1967, Pianka 1970, Solbrig and Simpson 1974), longevity and fecundity (Medawar 1952, Rose and Charlesworth 1980), reproduction and growth (Warner 1984, Reznick 1985, Bell and Koufopanou 1986), competitive ability and resistance to exploitation (Lubchenco 1978, Lenski and Levin 1985), and others. Indeed, trade-offs are central to almost all hypotheses concerning the evolution of generalists vs. specialists (Levins 1968, Huey and Hertz 1984, Jaenike 1990, Van Tienderen 1991).

Among these trade-off theories, the notion of *r* vs.

K selection has been perhaps the most historically prominent. *K* selection is presumed to optimize performance when a population is near its carrying capacity and resources are scarce, whereas *r* selection occurs when a population is sparse, resources are abundant, and its per capita growth rate is near its maximum (MacArthur and Wilson 1967, Pianka 1970, Roughgarden 1971, Mueller and Ayala 1981). Although most ecologists no longer think that this simple dichotomy provides an adequate classification for life history strategies (Stearns 1992), the more general hypothesis of an evolutionary trade-off in performance under conditions of resource scarcity vs. abundance remains widely held.

Like many of their counterparts who study animals and plants, microbial ecologists often assume that trade-offs in performance under conditions of resource abundance vs. scarcity are pervasive in the organisms that they study (Matin and Veldkamp 1978, Konings and Veldkamp 1980, Kuenen and Harder 1982, Veldkamp et al. 1984, Andrews and Harris 1986, Andrews 1991, Greer et al. 1992). The short generation times of microbes, their large population sizes, and ease of manipulation make them excellent model organisms for examining trade-off hypotheses such as this one. In-

deed, in a well-known set of experiments using the bacterium *Escherichia coli*, Luckinbill (1978, 1984) allowed populations to evolve under two distinct regimes that he thought differed substantially with respect to selection on growth parameters related to resource availability. However, other authors have recently argued that, in fact, the two selection regimes used by Luckinbill were not very different in their selective character, rendering moot his finding against any trade-off (Vasi et al. 1994). Thus, additional experiments are needed to address convincingly the question of whether there is an evolutionary trade-off in the competitive ability of bacteria at high vs. low resource levels. Addressing this issue in a rigorous manner not only is important for ecological theory, but also may be important for identifying those bacterial strains that are the most useful for bioremediation of polluted sites (National Research Council 1993). Can one find a generalist that will efficiently degrade a certain pollutant over a wide range of concentrations? Or must an array of specialists, each adapted to a different substrate concentration, be employed?

In principle, one could test the hypothesis of fitness trade-offs under conditions of resource scarcity vs. abundance by several different methodologies, such as examining the distribution of genetically distinct organisms across habitats that differ in resource availability or comparing relevant life history traits across populations or species. But both these approaches have inherent limitations that weaken the resulting inferences. In the former case, the complexity of ecological habitats and communities means that many variables other than just resource availability must also affect the distribution of organisms. Identifying, and then excluding, variables that confound the hypothesis at hand is, at best, a formidable task. The comparative method is similarly fraught with inferential limitations. These include the problem of distinguishing adaptive from phylogenetic explanations for differences among organisms (Harvey and Pagel 1991) as well as meaningless estimates of performance traits due to differential "pre-adaptation" of organisms to the arbitrary laboratory environment in which they are measured (Mueller and Ayala 1981, Service and Rose 1985). An experimental evolutionary approach circumvents these difficulties. First, ecological conditions are precisely controlled to minimize confounding selective factors between replicates and treatments. Second, comparisons are made between organisms that have known and direct ancestor–descendant relationships. Also, replication of evolutionary treatments allows the extent of heterogeneity in response to selective conditions to be quantified and included in the statistical analysis (e.g., Bennett et al. 1992). Finally, performance traits are measured in environments that correspond to an organism's most recent evolutionary history, except for the specific variable of interest that is manipulated. Therefore, we have used an experimental evolutionary approach to test the hypothesis of a trade-off in performance under ecological conditions of resource scarcity vs. abundance. Populations of bacteria were propagated under each type of condition for many generations, and changes in the relative fitness of the derived strains were then measured by competition experiments, both in the environment in which they had evolved and in the alternative environment. If fitness gains in the selective environment usually correspond with losses in the alternative environment, this would support a fundamental role for trade-offs in adaptation to ecologically distinct environments.

OVERVIEW OF THE EXPERIMENTAL DESIGN

The evolution experiments were performed under two different selective regimes and in two stages (Fig. 1). One regime was batch culture, in which bacterial populations were serially diluted each day to low density in fresh medium, where they then grew at or near their maximum growth rate for several generations before the limiting resource was depleted. Under this regime, the populations received high concentrations of resource at regular intervals, and most population growth occurred during exponential phase. Natural selection would thus favor mutants that were better able to exploit the abundant resources. The other selective regime was chemostat culture, in which fresh medium flowed into the culture vessels at a constant rate; the bacterial populations maintained a high equilibrium density while holding the limiting resource to a low concentration. Hence, the bacteria constantly experienced high population density and low resource availability. Natural selection under these conditions would favor mutants that were better able to scavenge scarce resources.

We performed the evolution experiments in two stages. In stage I, two strains recently isolated from nature were used to found replicate populations that evolved for 75 d under either the batch or the chemostat regime (Fig. 1). We used these two different founding strains, designated F and S, because we were interested in whether bacteria that had been shaped by different forces in nature would respond similarly to the two regimes. Therefore, we chose one strain that had a high maximum growth rate (F) under laboratory conditions and another strain that grew much more slowly (S). In stage I, we had two replicate populations for both founding strains under each of two selective regimes, giving a total of eight populations.

During this first stage of experimental evolution, the sole source of carbon provided in both regimes was succinate, which is a central substrate in bacterial metabolism. Eight strains derived from stage I were then used to found a total of 32 new populations for stage II, with two replicates under each of the two culture regimes for every founder. During this second stage, the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was provided as the sole source of carbon. This design,
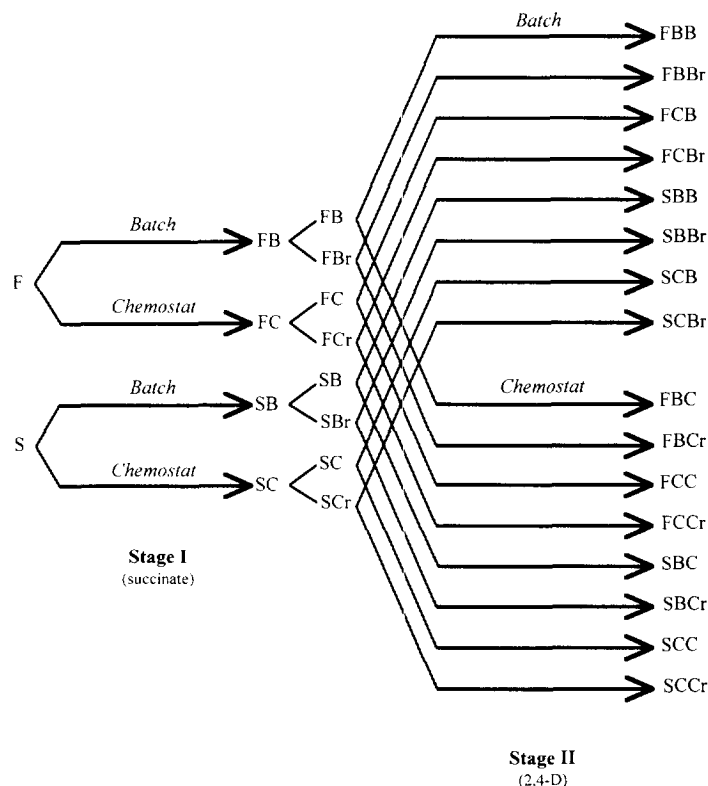
FIG. 1. Design of the experimental evolution, and derivation of the bacterial lines. Each horizontal line represents two independently evolved replicate populations. Nomenclature examples: F = fast growing ancestral strain; S = slow growing ancestral strain; FB = F-derived, stage I batch evolution; SC = S-derived, stage I chemostat evolution; FCB = F-derived, stage I chemostat, stage II batch; r = streptomycin resistant strain. Stage I was performed in medium containing succinate as sole carbon source and stage II in medium containing 2,4-D as sole carbon source; each stage lasted 75 d. See *Introduction: Overview of the experimental design* for a summary of the essential features.

with two successive stages of evolution using different resources, allowed an examination of the specificity of adaptation with respect to substrate type (Velicer 1999) as well as adaptation to substrate concentration. However, this paper is concerned only with the latter (adaptation specific to substrate availability). For present purposes, the two evolutionary stages thus can be regarded as two separate experiments. That is, each derived strain competed against its *proximate* ancestor (stored in a freezer and marked genetically so that the competitors could be readily distinguished) and for the substrate on which the derived strain was most recently selected. Competition assays were performed under both the batch and chemostat regimes to test the trade-off hypothesis, which predicts that genotypes that became better adapted to one regime simultaneously became inferior competitors under the alternative regime.

MATERIALS AND METHODS

Bacterial strains and culture media

The two ancestral strains used in this study, originally designated TFD3 and TFD13, were isolated from sludge in Oregon and soil in Michigan, respectively (Tonso et al. 1995). Both are able to catabolize 2,4-D, and both belong to the genus *Burkholderia* in the β2 subgroup of the Proteobacteria based on their 16S rDNA sequences (McGowan 1995). These two strains were chosen for this study because one of them (TFD3)

grows relatively fast, whereas the other grows more slowly. Henceforth, TFD3 and TFD13 are called "F" (fast) and "S" (slow), respectively. The maximum growth rates in succinate for F and S are 0.42 h^{-1} and 0.09 h^{-1}, respectively. In 2,4-D medium, the maximum growth rates for F and S are 0.21 h^{-1} and 0.08 h^{-1}, respectively. F and S were stored as clones in medium containing 10% glycerol at $-80°C$.

All experiments were performed in the same mineral-salts base medium, in which each liter contained 1.71 g K_2HPO_4, 0.3 g Na_2PO_4, 0.33 g $(NH_4)_2SO_4$, 0.246 g $MgSO_4·7H_2O$, 0.12 g $Na_2EDTA·2H_2O$, 20 mg NaOH, 4 mg $ZnSO_4·7H_2O$, 3 mg $MnSO_4·H_2O$, 1 mg $CuSO_4·5H_2O$, 30 mg $FeSO_4·7H_2O$, 52 mg Na_2SO_4, and 1 mg $NaMoO_4·2H_2O$. As a source of carbon, this medium was then supplemented with either succinate or 2,4-D at a concentration of 500 mg/L. All cultures were maintained at a constant temperature of 25°C.

Stage I batch evolution

F and S were each used to establish two replicate lines by inoculating 10-mL flasks of succinate medium. Upon reaching stationary phase, the lines were transferred into fresh medium and this transfer cycle was repeated daily for 75 d. The dilution factors were 1/100 (0.1 mL into 9.9 mL) for the F lines and 18/100 (1.8 mL into 8.2 mL) for the S lines, which allowed 6.64 ($=\log_2 100$) and 2.47 ($=\log_2 100/18$) generations of bi-

nary fission per day, respectively, giving ~500 and 185 generations of evolution in total for the F and S descendants, respectively. This difference in dilution factor (and hence number of generations per day) between strains was necessary to prevent the faster growing lines from spending a much larger portion of their daily cycle in stationary phase than the slow growers. Upon completing this experiment (and all of the evolution experiments in this study) clones were chosen at random from each population and stored at −80°C for use in subsequent competition experiments (described below) with their ancestral strains. The derived clones from stage I batch lines were designated FB1, FB2, SB1, and SB2 (where "B" stands for batch-evolved).

Stage I chemostat evolution

F and S were used to establish two replicate lines each in chemostat vessels that contained succinate medium. Vessels for the F lines were maintained at 18 mL, while vessels for the S lines were maintained at 50 mL. The flow of fresh medium into each chemostat was kept at 2.5 mL/h, setting growth rates at 0.139 h^{-1} for the F lines and 0.050 h^{-1} for the S lines. These realized rates are ~33% and 56% of the maximum rates on succinate for F and S, respectively. Because every derived strain competed against its own ancestor, and in its own regimes, the important point is not to make the experiments absolutely identical for the F and S lines, but rather to ensure that the two regimes provide very different resource availabilities and hence growth rates for each strain. It is also not important that the number of generations be identical across strains or even regimes, because the trade-off hypothesis makes clear and simple directional predictions. (See also the Discussion.) The F lines experienced 75 d of evolution (~360 generations), whereas the S lines were stopped after 73 d (~125 generations) due to blockage of the nutrient input. Derived clones from stage I chemostat lines are called FC1, FC2, SC1, and SC2 (where "C" stands for chemostat-evolved).

Genetic markers

To exclude the possibility of contamination, we compared DNA "fingerprints" from the stage I (and II) derived clones with those of the ancestral strains F and S. Specifically, we examined electrophoretic patterns obtained from REP-PCR amplification products (Versalovic et al. 1991) and found no discrepancies, indicating that all of the evolved clones were, in fact, true descendants of the ancestral strains.

To perform competition experiments, we needed a genetic marker that would allow us to distinguish two competing strains. To that end, we used selective plating to obtain streptomycin-resistant mutants of FB1, FC1, SB1, and SC1. These resistant clones were designated FBr, FCr, SBr, and SCr, and they too were stored at −80°C. In addition to being necessary for competition experiments, these resistant clones were used to

found one-half of the Stage II evolving lines. All of the derived clones from Stage II lines retained their appropriate streptomycin marker state.

Stage II batch evolution

Each of the eight clones FB1, FBr, FC1, FCr, SB1, SBr, SC1, and SCr was used to found two replicate populations by inoculating 10-mL flasks of 2,4-D medium. Transfers cycles were performed for 75 d, exactly as during stage I evolution, except that the medium contained 2,4-D (instead of succinate) and dilution factors were adjusted to ensure that each founding strain could replace itself during each cycle. The dilution factors were as follows: 1/100 for FB1, FBr, and FC1 (6.64 generations per day, ~500 generations total); 7.7/100 for FCr (3.70 generations per day, ~275 generations total); 20/100 for SB1 and SBr (2.32 generations per day, ~175 generations total); and 33.3/100 for SC1 and SCr (1.59 generations per day, ~120 generations total). As noted above, the important point was not to make the experiments absolutely identical for each group, which will be compared with its own ancestor in its own regimes, but rather to ensure that the batch and chemostat growth regimes are sufficiently distinct in terms of resource availability and demography. The sixteen derived clones were designated FBB1, FBB2, FBBr1, FBBr2, FCB1, FCB2, FCBr1, FCBr2, SBB1, SBB2, SBBr1, SBBr2, SCB1, SCB2, SCBr1, and SCBr2. Here, the first letter denotes the original ancestor; the second and third letters are the regimes during stages I and II, respectively; the letter "r" indicates that the line was founded by a streptomycin-resistant clone at the start of stage II; and the numerals distinguish the replicate populations.

Stage II chemostat evolution

The same eight clones (FB1, FBr, FC1, FCr, SB1, SBr, SC1, and SCr) were each used to found two replicate populations in chemostat vessels containing 2,4-D medium. Every population was grown until the medium appeared turbid before initiating the flow of fresh medium through the chemostat. In all cases, a flow rate of 2.0 mL/h was maintained for 75 d. Given this constant flow, chemostat volumes were adjusted so that each population grew at roughly half the founder's maximum rate in 2,4-D (details in Velicer [1997]), following the same logic as above wherein we sought to make the batch and chemostat regimes distinct for all founding strains. The volume used for FB1, FBr, and FC1 lines was 18 mL, which allowed growth at 0.111 h^{-1} (3.85 generations per day, ~290 generations total); the volume for the FCr lines was 36 mL, which permitted a growth rate of 0.056 h^{-1} (1.92 generations per day, ~145 generations total); and the volume for the SB1, SBr, SC, and SCr lines was 48 mL, yielding a rate of 0.042 h^{-1} (1.44 generations per day, ~110 generations total). The clones derived from Stage II chemostat lines were designated FBC1, FBC2, FBCr1,

FBCr2, FCC1, FCC2, FCCr1, FCCr2, SBC1, SBC2, SBCr1, and SCCr1. Four populations were lost due to contamination of the vessels, so there are no clones designated SBCr2, SCC1, SCC2, or SCCr2.

Batch competition assays

All competition assays (batch and chemostat) were performed in the same medium, and with the same dilution factor, that the particular derived strains had experienced. Thus, stage I competitions were performed in succinate medium, and stage II competitions were run in 2,4-D medium. For batch competitions, two clones were mixed together in the same flask, where they competed for a common pool of limiting nutrient. In all cases, the two competitors could be distinguished by a genetic marker. For stage I competitions, the ancestral F strain and each of the F-descendant clones competed against the streptomycin-resistant clone, FBr, while the ancestral S strain and all of the S-descendants competed against the streptomycin-resistant clone, SBr. For stage II competitions, one of the competitors was a descendant and the other was the proximate ancestral genotype carrying the reciprocal streptomycin marker. The proximate ancestors of the stage II descendants are the stage I derived clones that were used to establish the stage II lines. For example, streptomycin-sensitive clones FBB1 and FBB2 competed against streptomycin-resistant FBr, whereas FBBr1 and FBBr2 each competed against FB1.

Prior to being mixed, the two competitors were grown separately in the assay environment for 24 h (one transfer cycle) so they were comparably acclimated. The two competitors were then mixed in fresh medium and this mixed population was propagated for one or more transfer cycles, depending on the relative fitnesses of the competitors. Longer experiments give better resolution of small changes in relative abundance, whereas shorter experiments are needed when competitive exclusion occurs so rapidly that one strain becomes too rare to quantify accurately. Initial ratios of the two competitors were also adjusted to permit accurate quantification of changes in relative abundance. Initial and final densities of each competitor were obtained by diluting the mixture on both selective and nonselective agar plates (with and without streptomycin, respectively). After adjusting for dilution prior to plating, the density of the resistant competitor was obtained directly from the colony count on the selective agar; the density of the sensitive competitor was estimated from the difference in density between the nonselective and selective plates. Details of the initial mixing ratio, daily dilution factor, and number of transfer cycles for each set of batch competition assays are reported in Velicer (1997).

For each pair of competitors, we performed multiple assays, each yielding an independent estimate of the selection rate constant, which provides a simple measure of the rate at which one competitor excludes an-

other. Experiments were performed in blocks of five pairwise matches, with five replicates per match, giving a total of 25 competitions per block. (One or two replicates were occasionally discarded due to contamination or insufficient plate counts.) For stage I, one block had F, FB1, FB2, FC1, and FC2 each competing against FBr, and a second block had S, SB1, SB2, SC1, and SC2 each competing against SBr. For stage II, we ran eight blocks, each consisting of one competition between the two reciprocally marked ancestors (e.g., FB1 vs. FBr) and four competitions between a set of derived clones and their reciprocally marked ancestor. For example, one block had pairings of FB1 vs. FBr, FBB1 vs. FBr, FBB2 vs. FBr, FBBr1 vs. FB1, and FBBr2 vs. FB1. (The blocks for the SBC and SCC sets were somewhat smaller because some of the derived lines were lost due to contamination.)

Calculation of selection rate constants in batch competitions

For each competitor, we calculated its realized Malthusian parameter, m_i, as its net rate of increase adjusted for the daily transfer and dilution into fresh medium:

$$m_i = (1/t)\ln[D^t \times N_i(t)/N_i(0)] \qquad (1)$$

where D is the dilution factor, t is the number of daily transfer cycles that the competition ran, and $N_i(0)$ and $N_i(t)$ are initial and final population densities (expressed at the same points in the growth cycle). The relative performance of two competitors is expressed as the selection rate constant s_{ij}, which is defined as the difference in their realized Malthusian parameters as they compete for a common pool of nutrients (Lenski et al. 1991, Travisano and Lenski 1996):

$$s_{ij} = m_i - m_j. \qquad (2)$$

Because the same factor D^t appears in the calculation of each competitor's Malthusian parameter, it drops out of the equation for the selection rate constant. Eq. 2 can be rearranged as

$$s_{ij} = (1/t)(\ln[N_i(t)/N_i(0)] - \ln[N_j(t)/N_j(0)]). \qquad (3)$$

The selection rate constant can thus be expressed as either the difference in two competitors' realized Malthusian parameters or the rate of change in their relative abundance, because these expressions are mathematically equivalent. Any difference in the plating efficiencies of the two competitors does not affect the calculation of the selection rate constant, provided only that the plating efficiencies are the same in the initial and final samples.

The effects of the streptomycin-resistance marker on performance were factored out of the values presented in the *Results* section, so that $s > 0$ indicates superior performance by a derived clone relative to its proximate ancestor, whereas $s < 0$ indicates that the derived clone is less fit than its ancestor. For example, to obtain the selection rate constant for derived clone FBB1 rel-

TABLE 1. Results of competition between ancestral bacteria and lines derived from stage-I evolution in medium containing succinate as sole carbon source.

Evolved line	Selected in	Selection rate, s (d^{-1})	
		Selection regime	Alternative regime
FB1	Batch	0.0260	0.0472
FB2	Batch	0.0910	−0.0374
FC1	Chemostat	**2.8721**	−0.3093
FC2	Chemostat	**2.0268**	**0.8125**
SB1	Batch	**0.2531**	0.3715
SB2	Batch	**0.3759**	**0.6026**
SC1	Chemostat	0.1729	**0.2379**
SC2	Chemostat	0.2521	0.0897

Notes: Selection rate constants, s, of stage I derived lines (relative to the original ancestors) are presented for both the selection and alternative regimes. Positive values indicate competitive superiority relative to the ancestor, whereas negative values indicate inferiority. Selection rate constants shown in bold are significantly different from the ancestral values (two-tailed t tests, $P < 0.05$).

TABLE 2. Results of competition between bacteria proximately ancestral to stage-II evolution in medium containing 2,4-D as sole carbon source and lines derived from stage-II evolution.

Evolved line	Selected in	Selection rate, s (d^{-1})	
		Selection regime	Alternative regime
FBB1	Batch	**0.4026**	**0.1821**
FBB2	Batch	**0.9675**	0.2162
FBBr1	Batch	**0.3116**	**0.2268**
FBBr2	Batch	**0.3439**	−0.4448
FCB1	Batch	0.4171	0.3177
FCB2	Batch	**0.6390**	−0.2586
FCBr1	Batch	**0.6788**	0.0150
FCBr2	Batch	−0.6600	−0.3296
FCC1	Chemostat	0.2341	**0.3291**
FCC2	Chemostat	**1.2510**	**0.6807**
FCCr1	Chemostat	**0.9161**	**−0.4631**
FCCr2	Chemostat	**0.7944**	−0.0517
FBC1	Chemostat	0.1432	−0.1468
FBC2	Chemostat	0.2944	−0.2777
FBCr1	Chemostat	**0.3265**	−0.3362
FBCr2	Chemostat	**0.6090**	−0.3798
SBB1	Batch	0.0379	−0.0154
SBB2	Batch	0.1073	**0.1013**
SBBr1	Batch	**0.2230**	−0.0151
SBBr2	Batch	0.5401	0.0098
SCB1	Batch	**0.6924**	0.2494
SCB2	Batch	**0.7670**	0.0758
SCBr1	Batch	**0.4899**	−0.2048
SCBr2	Batch	**0.3680**	−0.4172
SCCr1	Chemostat	0.0013	0.0925
SBC1	Chemostat	**0.0701**	0.1207
SBC2	Chemostat	0.0026	0.0031
SBCr1	Chemostat	**0.0763**	0.0845

Notes: Selection rate constants, s, of stage II derived lines (relative to their proximate ancestors) are shown for both the selection and alternative regimes. Positive values indicate competitive superiority relative to the ancestor, whereas negative values indicate inferiority. Selection rate constants shown in bold are significantly different from the ancestral values (two-tailed t test, $P < 0.05$).

ative to its proximate ancestor FB1, each one separately competed against the marked clone FBr. The selection rate constant of FB1 relative to FBr was then subtracted from that of FBB1 relative to FBr to obtain the standardized selection rate constant of FBB1 relative to FB1.

Chemostat competition assays

Chemostat competition assays were performed using the same procedures as for the batch competition assays, except as indicated here. Prior to each assay, both competitors were grown to stationary phase in flasks containing the same medium in which they would compete. The two competitors were then mixed in a chemostat vessel at an initial ratio of 100 of the streptomycin-sensitive competitor to 1 of the resistant competitor, at which time the flow of medium through the vessel was started. To allow acclimation to the chemostat environment, the mixed population grew for at least 12 h before the first sample was taken. Each vessel was sampled and plated several times, on selective and nonselective agar, to monitor changes in the densities of the two competitors over a period of 6–10 d. The pairwise matches and block design of the chemostat competitions were the same as those for the batch competitions, except that only two replicates (rather than five) were run for each pair of competitors. Although we ran fewer replicates of the chemostat competitions than of the batch competitions, each replicate of a chemostat competition included more internal time points.

The selection rate constant for each replicate was calculated as the slope of the regression of the natural logarithm of the ratio of the two competitors—$\ln[N_i(t)/N_j(t)]$—versus time. This slope is therefore equivalent to s_{ij} in Eq. 3. As with the batch competitions, the selection rate constants were adjusted for the effects

of the streptomycin-resistance marker, so that $s > 0$ indicates superior performance by a derived clone relative to its proximate ancestor, while $s < 0$ indicates that the derived clone is less fit than its ancestor.

RESULTS

Adaptation to selective regime

We first examined the change in performance of each derived line in the environment in which it was selected. Including both stages, a total of 36 independently derived lines were thus examined. For each line, we performed a two-tailed t test comparing the replicated estimates of the selection rate constants for the derived clone and the corresponding marker control. The first numerical columns in Tables 1 (stage I) and 2 (stage II) show the resulting selection rate constant for the derived clone, adjusted for the marker control. Values shown in boldface are significant at $P < 0.05$.

Of the 36 lines, 22 showed significant adaptation to their own selective regime; none of them showed a significant loss of performance in the selective environment. In four sets (FC, SB, FBB, and SCB), all of the replicate lines improved significantly, whereas most sets include some lines that showed significant improvement and others that did not. Of the 14 lines that did not show significant adaptation, the values of the selection rate constant, s, were positive in every case except one. This asymmetry strongly suggests that some other lines also adapted to their selective regime, but we lacked sufficient statistical power to claim adaptation in every case.

Of the stage I lines derived from the fast-growing ancestral strain, F, neither batch line (FB1 or FB2) showed significant improvement in the batch regime, in which resources were plentiful and hence growth was fast (Table 1). However, both F-derived lines selected in the chemostat regime (FC1 and FC2) improved significantly in that environment, in which resources were scarce and growth therefore slower. Among the stage I descendants of the slow-growing ancestral strain, S, the opposite outcomes were seen. That is, both batch-selected lines (SB1 and SB2) adapted significantly to that fast-growth regime, but neither of the chemostat-selected lines (SC1 and SC2) showed significant improvement in that slow-growth environment. Thus, the derived lines showed the greater improvement under the regime where the ancestor was weaker.

Such historical effects are less consistent among the stage II lines. The S-derived lines did, in fact, tend to adapt more to novel environments than to a continuation of their previous environment. Of the seven lines that switched from one regime to another (SCB and SBC sets), six showed significant adaptation to the new regime. By contrast, only one of five lines that stayed in the same environment (SBB and SCC sets) showed further adaptation to that regime. These results are thus consistent with the notion that it is easier to improve on weak performance than it is to enhance performance that is already strong. However, this pattern did not hold for the F-derived lines: seven of eight lines that stayed under the same regime (FBB and FCC sets) continued to improve, whereas only four of eight lines that switched regimes (FCB and FBC sets) showed significant adaptation to the new regime relative to their proximate ancestor.

Despite this ambiguous evidence for historicity, the main result is clear: the majority of lines adapted to the selective regime under which they evolved. The key question, therefore, is whether the lines lost fitness under the alternative regime, as predicted by the trade-off hypothesis.

Performance under alternative regime

Once again, we performed for each line a two-tailed t test comparing the estimates of the selection rate con-

stants, but now measured in the alternative regime, for the derived clone and its appropriate marker control. The second numerical columns in Tables 1 (stage I) and 2 (stage II) show the resulting selection rate constant for the derived clone, adjusted for the marker control, with boldface values denoting significance at $P < 0.05$. Let us first consider only those 22 lines that showed significant adaptation to their own regime. Of these 22 lines, seven (FC2, SB2, FBB1, FBBr1, FBBr2, FCC2, and FCCr1) also showed significant changes in their performance in the alternative regime. These seven lines therefore provide the clearest tests of the trade-off hypothesis. Only two (FBBr2 and FCCr1) show the pattern of evolutionary change predicted by the trade-off hypothesis, that is, significant improvement under the selective environment and a significant loss of performance in the alternative regime. This outcome is illustrated in Fig. 2a for FCCr1, which became less fit in the batch environment as it became more competitive in the chemostat environment. The other five lines improved significantly in both their selective and alternative regimes, contrary to the trade-off hypothesis. This outcome of correlated gain in the alternative regime is shown in Fig. 2b for FBB1, which became significantly more competitive in the chemostat even as it became better adapted to the batch regime.

These seven lines yield the clearest interpretation, and both individually and collectively they do not support the trade-off hypothesis (Table 3, row 1). Beyond these seven lines, any interpretation of the responses of individual lines is necessarily more ambiguous, but collectively they also do not support the trade-off hypothesis. Fifteen lines underwent significant adaptation to their selective regime, but did not show any significant changes in their performance under the alternative regime (Table 3, row 2). Eight of these responses to the alternative regime were negative (consistent with a trade-off) and seven were positive (inconsistent with a trade-off). Fourteen lines did not improve significantly in their selective regime, but as noted above most of these responses were in the direction expected if they too had adapted. Among these 14 lines, three showed significant changes in their performance in the alternative environment, all of which were improvements and hence inconsistent with the trade-off hypothesis (Table 3, row 3). Finally, the other eleven lines, which showed nonsignificant changes in both regimes, include five cases in which the correlated response was negative and six where it was positive (Table 3, row 4). In summary, there is no evidence of a trend that would support the trade-off hypothesis, nor do those lines that showed clear changes in their performance in both regimes typically exhibit any trade-off.

Homogeneous and heterogeneous responses to selection

Among the 36 lines taken together, it is clear there were a diversity of responses, some consistent with the

FIG. 2. Examples of outcomes that are consistent and inconsistent with the trade-off hypothesis. (a) Strain FCCr1 showed significant adaptation to its selective regime (chemostat) and significant loss of fitness under the alternative regime (batch), as predicted by the trade-off hypothesis. (b) Strain FBB1 showed significant adaptation to its selective regime (batch) but also improved under the alternative regime (chemostat), contrary to the trade-off hypothesis. Error bars indicate 95% confidence intervals.
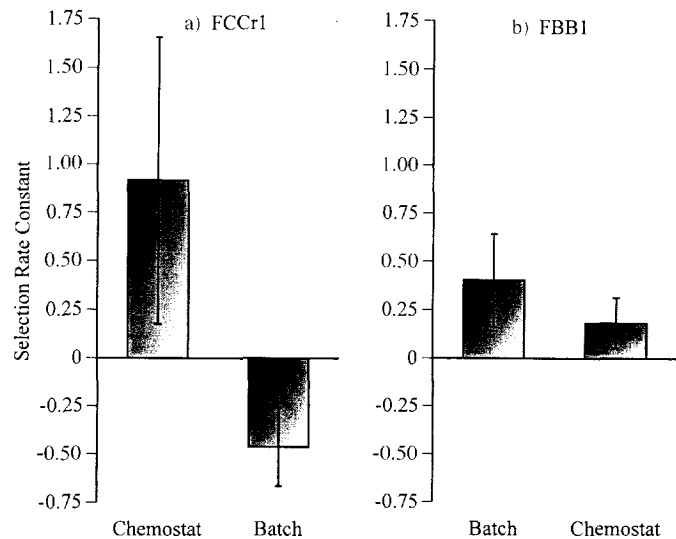
trade-off hypothesis and others inconsistent with it. In this section, we ask whether there are consistent patterns among various sets of lines. That is, might some sets consistently exhibit trade-offs whereas other sets show the opposite pattern?

With only two lines per set, the stage I sets do not provide much power for addressing this issue. We would only note that both the SB and SC sets were consistent insofar as both lines in each of these sets appeared to have improved in both environments. By contrast, the two lines in the FC set showed one case of correlated improvement and one case suggestive of a trade-off. Neither of the FB lines appears to have changed their performance much under either regime.

Among the six stage II sets with four lines, only the FBC set gave consistent responses among all of its constituent lines in both the selective and alternative environments (Fig. 3, □). All four lines appear to have improved in their selective chemostat regime, and all four appear to have lower fitness than their proximate

ancestor in the alternative batch regime. Many of these changes are not statistically significant when individual lines are compared separately to the ancestor. However, if the replicate lines are considered together, with each line as the unit of observation, then the set shows a significant improvement in the chemostat regime ($t = 3.533$, 3 df, 2-tailed $P = 0.0386$) and a significant loss of fitness in the alternative batch regime ($t = 5.632$, 3 df, 2-tailed $P = 0.0111$).

For the other Stage II sets, however, there are no consistent patterns of gain or loss of performance in the alternative regimes. In most sets, one or two lines appear to be worse in the alternative regime than their proximate ancestor, while the other two or three appear

TABLE 3. Frequency of adaptation patterns that are consistent and inconsistent with the trade-off hypothesis.

Performance	Comparison with tradeoff hypothesis	
	Consistent	Inconsistent
1) Significant responses in both selective and alternative regimes	2	5
2) Significant response in selective regime only	8	7
3) Significant response in alternative regime only	0	3
4) Significant response in neither regime	5	6

Note: The rows indicate the number of cases for each outcome, grouped according to whether the corresponding selection rate constants are significant (see Tables 1 and 2).
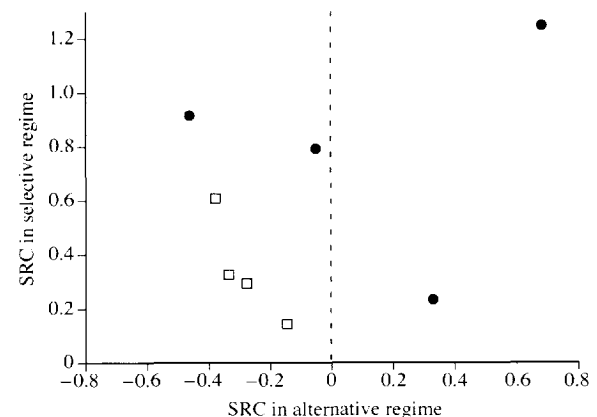

FIG. 3. Examples of homogeneous and heterogeneous correlated responses of replicate lines within a set. Most Stage II sets show a heterogeneous pattern of responses to their alternative regime, as exemplified by the FCC set (●), in which two lines improved while two other lines became worse in batch culture. The FBC set (□) shows a more homogeneous pattern, in which all four lines appear to have reduced fitness in the batch regime.

to have improved fitness. This is exemplified in Fig. 3 by the FCC set (●), in which two lines show improvement in the alternative (batch) regime and two either show or suggest a loss of fitness. Again, some of the individual lines in these sets are not significant, but the overall pattern strongly suggests that improved performance in a selective regime can be associated with either a loss or gain of fitness in the alternative regime.

DISCUSSION

Implications for the trade-off hypothesis

The hypothesis of a trade-off in the performance of bacteria under conditions of resource abundance vs. scarcity can be tested by either comparative or experimental approaches. To the best of our knowledge, the few systematic comparative tests of this hypothesis have failed to provide much support (Greer et al. 1992, Lenski et al. 1997, Velicer 1997), although some studies suggest that trade-offs are important for certain pairs of competitors (Matin and Veldkamp 1978, Kuenen and Harder 1982, Veldkamp et al. 1984), and it is also often assumed that such trade-offs exist (Andrews 1991). Greer et al. (1992) and Lenski et al. (1997) measured maximum growth rates (μ_{max}) and substrate affinities (μ_{max}/K_s) on 2,4-D for a number of natural isolates of bacteria able to degrade that substrate. These parameters govern growth rates at high and low substrate levels, respectively (Monod 1949). Neither study found any significant negative correlation between them, whereas such a correlation is predicted by the trade-off hypothesis. Velicer (1997) also directly measured the growth rates at high and low concentrations of two different substrates for seven bacterial strains isolated from a variety of locations and conditions. Whereas the trade-off hypothesis predicts a negative correlation between growth rates at high and low concentrations, the opposite trend was observed in both substrates, although the correlations were nonsignificant when the data were scaled to take into account phylogenetic relationships of the strains. Taken as a whole, the comparative studies neither strongly support nor definitely refute the hypothesis of a trade-off in bacterial performance under conditions of resource abundance vs. scarcity.

Using an experimental approach, Luckinbill (1978, 1984) sought to test certain predictions of r and K selection theory, including especially the existence of a trade-off in performance under alternative demographic regimes. Replicate populations of *Eschericia coli* were propagated under two different batch-culture regimes, which were supposed to promote adaptation to either low or high population density. Populations under one regime were kept in perpetual exponential growth by transferring them into fresh medium before they exhausted their resources, whereas populations under the other regime were allowed to exhaust their resources before they were transferred to fresh medium.

Derived populations showed significant genetic adaptation to their own selective regimes, and when the populations were then tested under the alternative regimes, it was observed that they had generally improved there, too. Thus, these experiments would seem to contradict the existence of the predicted trade-off.

However, a more recent analysis of selection gradients for demographic parameters in populations of *E. coli* propagated by serial batch-culture suggests that the two regimes imposed by Luckinbill may actually have been very similar in their selective effects (Vasi et al. 1994). In particular, although the bacteria periodically exhausted their resources in his high-density regime, almost all of the change in population numbers, and hence the opportunity for selection, would have occurred in the exponential-growth phase, just as it would have in the low-density regime. Thus, it appears that maximum exponential growth rate was the primary target of selection under both his low- and high-density selection regimes. Consequently, Luckinbill's results do not provide a definitive test of the hypothesized trade-off in performance under conditions of resource abundance vs. scarcity, even though his work showed clearly the potential power of experiments with microbial populations to address fundamental questions in evolutionary ecology. Vasi et al. (1994) also showed that selection acting on demographic parameters should be more distinct between a batch regime, in which most population growth occurs while resources are abundant, and a chemostat regime, in which all of the growth occurs while resources are scarce.

In this study, we propagated bacteria under batch and chemostat regimes in order to test the idea of evolutionary trade-offs in competitive fitness under conditions of resource abundance and scarcity. By virtue of using such distinct selective regimes as well as having extensive replication (including multiple ancestral strains), we believe that our study provides a more appropriate and powerful test of this trade-off hypothesis than have previous studies. Overall, our findings fail to support the hypothesis, and several specific results directly contradict it. Only two derived lines (FBBr2 and FCCr1) show definite trade-offs, having significantly adapted to their own selective regime while becoming significantly less competitive under the alternative regime. By contrast, five lines (FC2, SB2, FBB1, FBBr1, and FCC2) significantly improved their fitness under the alternative regime even as they became better adapted to their selective regime, in opposition to the trade-off hypothesis. Summing over all 36 derived lines in this study (including those for which the changes in performance were not significant), 15 support the trade-off hypothesis, whereas 21 show the opposite pattern (Table 3). Thus, neither specific cases nor overall patterns indicate the necessity, or even generality, of an evolutionary trade-off in bacterial competitive fitness under conditions of resource abundance vs. scarcity.

Levels of analysis and strain history

Our data can be analyzed at the level of either individual lines or treatment groups that are defined by some combination of strain history (Travisano et al. 1995) and selective regime. The primary question of interest for this study is whether the hypothesis of a trade-off in performance under conditions of resource abundance vs. scarcity is generally true. To address this general question, we asked whether observed changes in performance were consistent with the trade-off hypothesis for each of the 36 independently derived bacterial lines in this study. In essence, each derived line allows a test of the directional prediction that is made by the hypothesis concerning changes in performance relative to its own ancestral state but in a dissimilar environment. In fact, more significant cases went opposite to the trade-off hypothesis, five. than were consistent with it, two (Table 3, row 1). By contrast, the more basic hypothesis that the derived lines would adapt to their own selective regime was uniformly supported in all 22 cases that were significant (Tables 1 and 2). Thus, our study had ample power to detect adaptive evolution, but the outcome of that evolution did not generally, or even typically, support the trade-off hypothesis.

Our experimental design also permits an examination of variation within and among the various treatment groups. One purpose of replicating lines in each treatment was to observe the extent of variation in response by a particular ancestral strain to a specific selective regime. The two lines that showed significant trade-offs (FBBr2 and FCCr1) both belonged to sets in which at least one line showed the opposite pattern of significant correlated improvement (FBB1, FBBr2, and FCC2). This simple fact demonstrates that the descendants of a common ancestor are not constrained, even over short periods of evolution, to follow similar trajectories with respect to trade-offs in performance under conditions of resource abundance vs. scarcity.

A second point of having replicated lines within each treatment was to permit an analysis of differences in pattern of adaptation between treatments that differed by ancestor and selective regime within each stage of evolution, and by substrate between the two stages (Fig. 1). That is, in principle, we can compare the average change, including both direct and correlated responses, among the distinct sets of lines. In practice, however, the necessity of varying the number of generations across treatments confounds such an analysis. We will limit ourselves, therefore, to noting one pattern in the data that suggests an effect of strain history on subsequent adaptation. Specifically, the unique histories of progenitor strains F (fast-growing) and S (slow-growing) in nature appear to have influenced the relative extent of their adaptation to the batch and chemostat regimes during stage I of the experimental evolution. Neither F-derived line that was propagated in batch culture showed any significant improvement in that regime, whereas both chemostat lines showed significant adaptation. The opposite responses were seen for S-derived lines: both batch lines significantly adapted to that regime, whereas neither chemostat line showed any significant improvement in their environment. For whatever reason of their separate histories, F was already able to grow faster than S when resources were plentiful. Therefore, it seems that S had more room for improvement under the fast-growth batch regime than did F, whereas F had greater opportunity to adapt to the slow-growth chemostat regime than did S. This conclusion is somewhat compromised, however, because S-derived batch lines had more generations to adapt to their regime than did the S-derived chemostat lines (185 and 125 generations, respectively). Nonetheless, it appears that each strain's unique history might play some role in its subsequent evolutionary responses to various regimes. If so, then it becomes even more difficult to obtain compelling evidence for the generality of this (or any other) trade-off hypothesis.

Strengths and limitations of this study

The primary strength of this investigation relative to most previous tests of the trade-off hypothesis is that the evolution relevant to the conclusions of the study occurred in well-defined experimental conditions, rather than under ill-defined conditions in the wild. Therefore, we could compare directly the performance of ancestral and derived lines after the latter had evolved in, and adapted to, known and reproducible selective regimes. Consequently, one can be confident that differences between derived lines and their ancestors were produced by these regimes, rather than by fortuitous variation among extant populations in their preadaptation to the laboratory (Service and Rose 1985), confounding effects of phylogenetic nonindependence (Harvey and Pagel 1991) or unknown selective factors in nature.

Another strength of this study is the experimental design, which included: two ancestral strains with distinct demographic characteristics, two separate stages of evolution using different resources, independently evolving replicate populations in each treatment, and two very different selective regimes. Employing multiple ancestral strains, and multiple resources, permits a more robust test of the generality of the hypothesized trade-off. Most importantly, the two experimental regimes, batch and chemostat culture, are clearly distinct in terms of resource availability and thus the selective forces they impose on evolving populations. Another important feature of this study is that fitness comparisons between ancestral and derived lines were based on competitions, rather than on measurements of particular demographic traits that may or may not be the most important determinants of fitness. Also, our experiments used naturally occurring strains rather than

those with long laboratory histories, unlike most other selection experiments with bacteria (but see also Mikkola and Kurland [1992], Korona et al. [1994]). Finally, the ancestral strains in our study were able to degrade a widely used herbicide, and some of the derived lines were selected for enhanced performance on that substrate. Thus, our findings might be relevant to strategies for enhancing bioremediation of polluted sites (National Research Council 1993). In particular, our data imply that managers of bioremediation sites should not automatically reject the possibility of identifying strains that perform well across a wide range of substrate concentrations.

One complication of our study is the variation among sets of lines in the dilution rate that was imposed during their evolution. This variation was necessary to ensure strong and divergent selection under the two regimes, given the pre-existing differences among strains in growth rates. Thus, the batch and chemostat regimes were always distinct in terms of resource availability and demography, regardless of the founding strain for each population. For logistical purposes, all the evolution experiments had the same absolute duration, so that given the variation in dilution rate the various populations evolved for different numbers of generations. The amount of an evolving population's change will obviously tend to increase over time, so that the final magnitudes of the fitness gains and losses are not directly comparable across the treatments in our experiment, as noted above. Fortunately, the trade-off hypothesis makes clear directional predictions regarding the evolution of each population relative to its ancestor. Therefore, throughout the *Results*, we emphasized the direction and statistical significance of the observed evolutionary changes, but not their final magnitude. Another complication of our study was the necessity of using a genetic marker to distinguish ancestral and derived strains in the competition assays, and of adjusting the assay results for the effect of the marker on fitness. The marker's effect on fitness was measured in, and independently adjusted for, each regime and set of strains. Thus, the analyses in Tables 1 and 2 always reflect appropriately paired controls for the effect of the genetic marker on fitness.

In our view, the most important limitation of this study is that the evolution experiment lasted for only hundreds of generations, rather than for thousands or even tens of thousands of generations. Such is the reality of a dissertation project. Therefore, we cannot exclude the possibility that trade-offs in performance under conditions of resource abundance vs. scarcity would eventually emerge as each population approached its local adaptive peak. For example, a hypothetical population evolving under the batch regime for 2000 generations may substantially enhance its performance in that environment, while it simultaneously experiences a more modest improvement in the chemostat environment (Fig. 4). But evolution for another
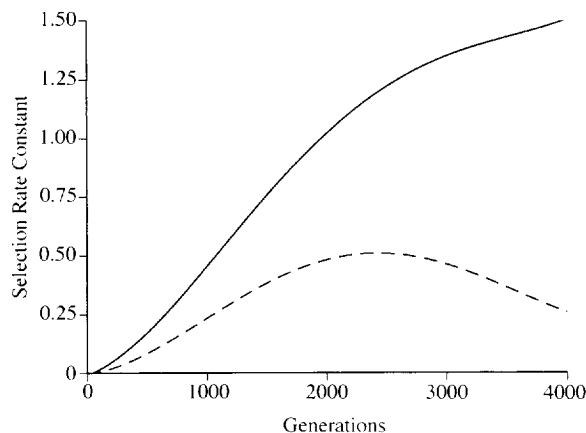

FIG. 4. Hypothetical threshold effect for a trade-off across two environments. Solid curve: the competitive fitness of an evolving population (measured by the selection rate constant relative to its ancestor) improves over time as it adapts to its environment (say, abundant resources). Dashed curve: the fitness of the same population in another environment (scarce resources) also improves for many generations, but eventually it declines as the population becomes more specialized. In this hypothetical example, there is no trade-off during the initial phase of adaptation to the selective environment, but a trade-off becomes manifest as the population encounters some constraint on simultaneous adaptation to the two environments.

2000 generations in batch culture might further improve its performance in that regime, while it begins to suffer a reduction in its chemostat fitness. Thus, the putative trade-offs may in fact exist, but only beyond a certain threshold level of adaptation to a particular regime. Below this threshold, correlated improvements in the alternative regime are possible and perhaps even likely. To demonstrate a trade-off threshold would require extending the experimental evolution for a long enough period that the potential for further adaptation to a given environment was exhausted (see Lenski and Travisano 1994). Despite this important limitation, our study clearly shows that bacteria are not invariably subject to trade-offs in performance over the entire evolutionary trajectory of adaptation to regimes characterized by either resource scarcity or abundance.

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