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Evolutionary Cheating in *Escherichia coli* Stationary Phase Cultures

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ABSTRACT

Starved cultures of *Escherichia coli* are highly dynamic, undergoing frequent population shifts. The shifts result from the spread of mutants able to grow under conditions that impose growth arrest on the ancestral population. To analyze competitive interactions underlying this dynamic we measured the survival of a typical mutant and the wild type during such population shifts. Here we show that the survival advantage of the mutant at any given time during a takeover is inversely dependent on its frequency in the population, its growth adversely affects the survival of the wild type, and its ability to survive in stationary phase at fixation is lower than that of its ancestor. These mutants do not enter, or exit early, the nondividing stationary-phase state, cooperatively maintained by the wild type. Thus they end up overrepresented as compared to their initial frequency at the onset of the stationary phase, and subsequently they increase disproportionately their contribution in terms of progeny to the succeeding generation in the next growth cycle, which is a case of evolutionary cheating. If analyzed through the game theory framework, these results might be explained by the prisoner's dilemma type of conflict, which predicts that selfish defection is favored over cooperation.

FEAST-AND-FAMINE is the most common lifestyle in the microbial world. Bacteria have evolved systems that enable them to use nutrients very efficiently, sustaining high growth rate, as well as to survive in the absence of growth, reflecting the importance of both phases for their survival. The feast-to-famine transition is not merely a response to a drop in nutrient availability; this transition also involves cell-to-cell signaling pathways, the results of which range from sporulation to fruiting body and complex pattern formation (Shapiro and Dworkin 1997; Shimkets 1999). In each of these cases intricate genetic circuits are set in motion, shutting off many genes needed for vegetative growth and inducing dozens of others. During this time, cells shift usage of still available resources into this energetically costly process.

Depending on the conditions, *Escherichia coli* is known to undergo different developmental programs resulting in social behaviors such as swarming motility or biofilm formation (Harshey and Matsuyuma 1994; Pratt and Kolter 1998), but they are not linked with the cessation of growth. However, its physiological state known as stationary phase bears many functional similarities to the starvation-induced phenomena mentioned above. The hallmark of stationary-phase cultures is that their constituent cells cease to grow and divide and display drastically reduced metabolic activity and increased resistance to many environmental stresses (Huisman *et al.* 1996; Hengge-Aronis 2000). In that view stationary-phase cells are functional equivalents of spores. The cessation of growth as *E. coli* cells enter stationary phase is governed in large part by the transcriptional regulator σ^s, which positively or negatively affects the expression of >50 genes (Loewen *et al.* 1998). The activity of σ^s itself is regulated at the transcriptional and translational level as well as at the level of protein stability (Hengge-Aronis 2000). Such complex regulation allows the integration of a multitude of intracellular (Huisman and Kolter 1994; Goodrich-Blair and Kolter 2000) and extracellular signals (Baca-DeLancey *et al.* 1999; Lazazzera 2000; Liu *et al.* 2000). Self-produced extracellular signals are particularly important modulators of gene expression that are responsible for the responses on the population level, *i.e.*, group responses. The major determining factor for the induction of σ^s response is the amount of nutrients (Notley and Ferenci 1996) and also the number of cells present to use them (Liu *et al.* 2000). Hence, the cells are able to respond as a population to changing environmental conditions and accomplish a coordinated transition into stationary phase. Therefore, entering stationary phase can be viewed as yet another example of a developmental program, resulting in a spore-like state in which cells, using minimal amounts of nutrients still present, can survive long periods of time, enduring many environmental assaults before resuming growth once the conditions change again.

As with any system depending on coordination, this transition is vulnerable to cheaters that do not perform a typical response (*e.g.*, cells that fail to respond to cell-to-cell signals or cells that fail to halt cell division) but

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profit in some way from the fact that other members of the population still respond. The strategic possibilities inherent in such a situation can be described within the framework of evolutionary game theory (Axelrod and Hamilton 1981; Maynard-Smith 1982; Sigmund 1995).

To interpret the dynamics of a stationary-phase culture from the perspective of evolutionary game theory, population members can be seen as the "players," the inheritable characteristics are the "strategies," and the "payoff" is fitness or survival success. Two contrasting strategies are available: cooperation and defection. The reference fitness/survival success (arbitrarily set as equal to 1) is that of two cooperators reaping the benefit of mutual cooperation, usually referred to as "reward" (R). An interaction between cooperator and defector lowers the fitness of the former (by the amount s_1) and increases the fitness of the latter (by the amount s_2); in other words the cooperator is left with the "sucker's payoff" (S, equal to $1 - s_1$) whereas the defector gains increased payoff, termed "temptation to defect" (T, equal to $1 + s_2$). Finally, mutual defection is costly to both defectors. Their fitness, decreased by cost c, is called "punishment" (P, equal to $1 - c$). The relative values of reward, temptation, sucker's payoff, and punishment thus define the outcome of the interaction, *i.e.*, whether cooperation or defection will be favored in a given system. The case in which $T > R > P > S$ is called the "prisoner's dilemma" and predicts the evolution of selfishness (defection) because regardless of the opponent's strategy it always pays off to cheat. However, a resulting all-defector population has lower fitness than the original population, which is an evolutionary paradox if one assumes that the result of natural selection is always an increase in fitness.

In reference to *E. coli* this would mean that cheaters would inevitably be found in each stationary phase population, given sufficient time, population size, and mutation rate, under the assumption that the maintenance of a stationary-phase state is indeed a cooperative behavior. It was shown that stationary-phase cultures of *E. coli* are easily invaded by mutants that outcompete the ancestral population (Zambrano *et al.* 1993). Such takeovers happen in all media and conditions tested thus far (Finkel *et al.* 1997). These mutants, expressing the *g*rowth *a*dvantage in *s*tationary *p*hase (GASP) phenotype, are able to grow when the ancestral population is in a state of no growth. The GASP mutants appear to be able to reinitiate growth by scavenging nutrients released by dying cells (Zinser and Kolter 1999). This can be regarded as "cheating" because these mutants ignore the signals that keep the rest of the wild-type population in the nondividing state and instead profit by growing, either by not fully inducing the starvation response or by reversing that response too early, resuming vegetative growth. Multiple rounds of takeovers happen during prolonged starvation (Finkel and Kolter 1999; Zinser and Kolter 1999). In rich Luria-Bertani (LB) medium the first round of GASP mutations are, in the majority of cases, reduced-function alleles of *rpoS*, which encodes σ^s, that result in an attenuated expression of the σ^s regulon. In subsequent takeovers mutations are more heterogeneous, but all appear to enhance amino acid catabolism and hence accelerate growth on amino acids released by dead cells (Zinser and Kolter 1999). When nutrient concentrations drop below critical levels (Notley and Ferenci 1996) and cell density reaches a threshold level (Liu *et al.* 2000), induction of the σ^s regulon directs the population along a strategy of growth cessation and development of stress resistance rather than continued scavenging and growth (Lazazzera 2000). Therefore, it makes sense that the majority of mutants able to ignore that course of action are mutants in the master-regulator σ^s.

To determine whether growth and scavenging, as opposed to persistence and stress resistance, in stationary phase can be defined as cheating, we measured the effect on stationary-phase survival these opposing strategies confer.

MATERIALS AND METHODS

Culture conditions and competition assays: Bacterial cultures were grown in LB rich medium [3 ml, mean total colony-forming unit (CFU) count after 24 hr of incubation $\approx 1.5 \times 10^{10}$/culture] in 18 × 15-mm glass test tubes at 37° with aeration. The strains we used were all derivatives of *E. coli* K-12 ZK126 (W3110 *tna2* Δ*lacU169*). Markers used to distinguish the competitors in mixed-culture experiments were either Val^r (valine-resistant growth on glucose) or Bgl^+ (growth on β-glucosides), previously shown (Zinser and Kolter 1999) and confirmed here to be neutral in our experimental conditions. ZK2552 and ZK2553 carry the *rpoS819* GASP allele (Zambrano *et al.* 1993) and Val^r and Bgl^+ markers, respectively. ZK2556 and ZK2557 carry the *rpoS* wild-type allele and Val^r and Bgl^+ markers, respectively. Competitors were grown for 24 hr prior to mixing. CFUs of each competitor were followed for 7 days by plating appropriate dilutions on selective agar plates (M63 0.2% glucose, 0.02% valine or M63 0.2% salicin). For each initial frequency of the GASP strain (Figure 2A), competitions were repeated at least four times. For each initial frequency of the GASP or wild-type strain, competitions were repeated at least three times (Figure 2, B and C). All competitions were repeated, reversing the markers at least twice, with the same results (data not shown). The rate of change in viable counts was estimated by $\ln(N_{d5}/N_{d3})/2d$, N_{d5} and N_{d3} being viable population densities at days 5 and 3, respectively.

Maximal cell density measurement: Overnight cultures of ZK2552, ZK2553, ZK2556, and ZK2557 grown in parallel in LB were diluted 500-fold into fresh LB in triplicate and grown as described above. After 12 hr, appropriate dilutions were plated on LB agar plates and CFU counts were calculated after 20 hr of incubation at 37°. The mean value from the triplicate measurements was calculated for each experiment; data presented are the mean of these triplicate averages across several experiments. CFU counts of wild type relative to GASP strain after 8 and 24 hr were the same (data not shown).

Survival in spent medium: Wild-type strain cultures were grown in LB medium as described above. After a 3-day incubation cells were removed by centrifugation. To remove any remaining cells the supernatant was sterilized by passing

through a Corning 0.2-μm pore syringe filter. In our experience compounds used for filter coating can end up in the pass-through and support bacterial growth to some extent, so to prevent such an interference, filters were extensively washed with water prior to use. One-day-old cultures of wild type and GASP strain were used to inoculate 3 ml of spent medium by diluting them 10,000-fold. These cultures were incubated at 37° with aeration and CFU counts were followed for the next 2 days by plating on either M63 0.2% glucose or LB agar plates. As a control, the same experiments were done with 1000- and 100,000-fold initial dilutions (data not shown). The rate of change in viable counts was estimated by $\ln(N_{d2}/N_{d0})/2d$, N_{d2} and N_{d0} being viable population densities at inoculation and 48 hr later, respectively.

Acid resistance: Mixed cultures of wild type and the GASP strain (150:1) were set up as described above. After 4 days of incubation, when CFU counts of both competitors reached ~1:1 ratio, cultures were assayed for acid resistance (the definition of acid resistance was based on that of Gorden and Small 1993). Cultures were diluted 100-fold in fresh LB/HCl pH 2.5, serial dilutions were plated on selective agar plates immediately as well as 2 hr after incubation at 37°, and CFU counts were calculated. As controls, the same experiments were done with a switch in the markers used to distinguish competitors, with 1500:1 initial ratio of wild type to the GASP strain, and using 24-hr-old pure cultures of wild type and GASP enumerated on LB agar plates (data not shown).

RESULTS AND DISCUSSION

In nature bacteria only rarely meet conditions able to support unrestricted growth; therefore, survival between consecutive growth phases is crucial for their survival in general. Different bacteria have developed various strategies to survive when nutrients are not available, many of which involve varying degrees of coordination. *E. coli* undergoes a complex developmental program resulting in nondividing cells highly resistant to various stresses. However, unlike real endospores these cells retain some metabolic activity, so the transition into this state should occur before nutrients become completely exhausted. It follows that both the transition into and the maintenance of stationary phase in *E. coli* should be coordinated. This is supported by the fact that the induction of σ^s regulon, the major determinant of starvation response, occurs at a nutrient concentration that is low but not zero (Notley and Ferenci 1996) and is also dependent on cell density (Liu *et al.* 2000). Another line of evidence is that only mutants, most of which bear a mutation in the gene encoding σ^s itself, are able to grow during long-term incubation in stationary phase (Zambrano *et al.* 1993). These mutants having attenuated expression of the σ^s regulon continue scavenging and growing as opposed to maintaining a highly resistant nongrowing state. In other words only deregulation of RpoS function allows for growth resumption under stationary-phase condition. This could be an example of evolutionary cheating because by outcompeting wild type in stationary phase these mutants (GASP mutants) increase their number disproportionately in the surviving population and in the extreme case can be the only ones to reach the next phase of nutrient abundance and growth.

To establish whether these opposing survival strategies in long-term stationary-phase cultures of *E. coli* (persistence and resistance *vs.* scavenging and growth) could be interpreted as cooperation and defection, respectively, we set up a system to analyze their effect on stationary-phase survival. Before presenting the results we emphasize the differences between our system and measurements we made from the standard ones used in experimental evolution.

The standard way of estimating relative fitness experimentally is comparison of the growth rates of the two competitors. However, because in stationary-phase cultures only GASP mutant cells grow and divide whereas wild-type cells either do not grow or die, this is not applicable. The relevant parameter in the stationary-phase state is the ability of semidormant cells to reach the next reproductive phase, when conditions will be propitious for growth. In that view a parallel can be drawn between sporangium or fruiting body containing dormant spores and the whole stationary-phase culture consisting of the cells in a spore-like state. By analogy, GASP mutants would be developmental mutants that end up overrepresented among spore-like cells as compared to their initial frequency at the onset of the stationary phase, and subsequently they would increase their contribution disproportionately in terms of progeny to the succeeding generation in the next growth cycle. Therefore, we compared the survival of two competitors in mixed cultures (competitors were allowed to reach stationary phase separately) at two different time points during prolonged incubation in stationary phase. We calculated the rate of change in viable counts, "survivorship rate," measuring the net effect of death and residual growth during the chosen time period. Positive and negative values of this survival parameter reflect, respectively, overall increase and decrease in viable counts.

We used a wild-type *E. coli* and a previously isolated GASP mutant. GASP strains carrying *rpoS819*, coding for an attenuated σ^s (Zambrano *et al.* 1993), and wild-type strains carrying the *rpoS*$^+$ allele, otherwise isogenic, were marked by neutral markers, which allowed us to follow them in mixed cultures.

We ran several series of mixed-culture experiments: (i) a GASP minority mixed with wild-type majority; (ii) a GASP minority mixed with a GASP majority carrying a different neutral marker; and (iii) a wild-type minority mixed with a wild-type majority carrying a different neutral marker. In each series of experiments competitors were mixed at several initial ratios, with the total number of cells kept constant. Competitions were done in the LB medium in which the GASP strain carrying the *rpoS819* allele was originally isolated. Typical results from mixing experiments are shown in Figure 1, where the minority strain was introduced as a 150-fold minority relative to

the competitor. In the case of GASP/GASP and wild-type/wild-type mixes (Figure 1, B and C) the dynamic of the minority and majority populations is the same, whereas in the case of the GASP/wild-type mix (Figure 1A) both populations first decline; but by day 3 the GASP mutant grows and by day 5 it takes over.

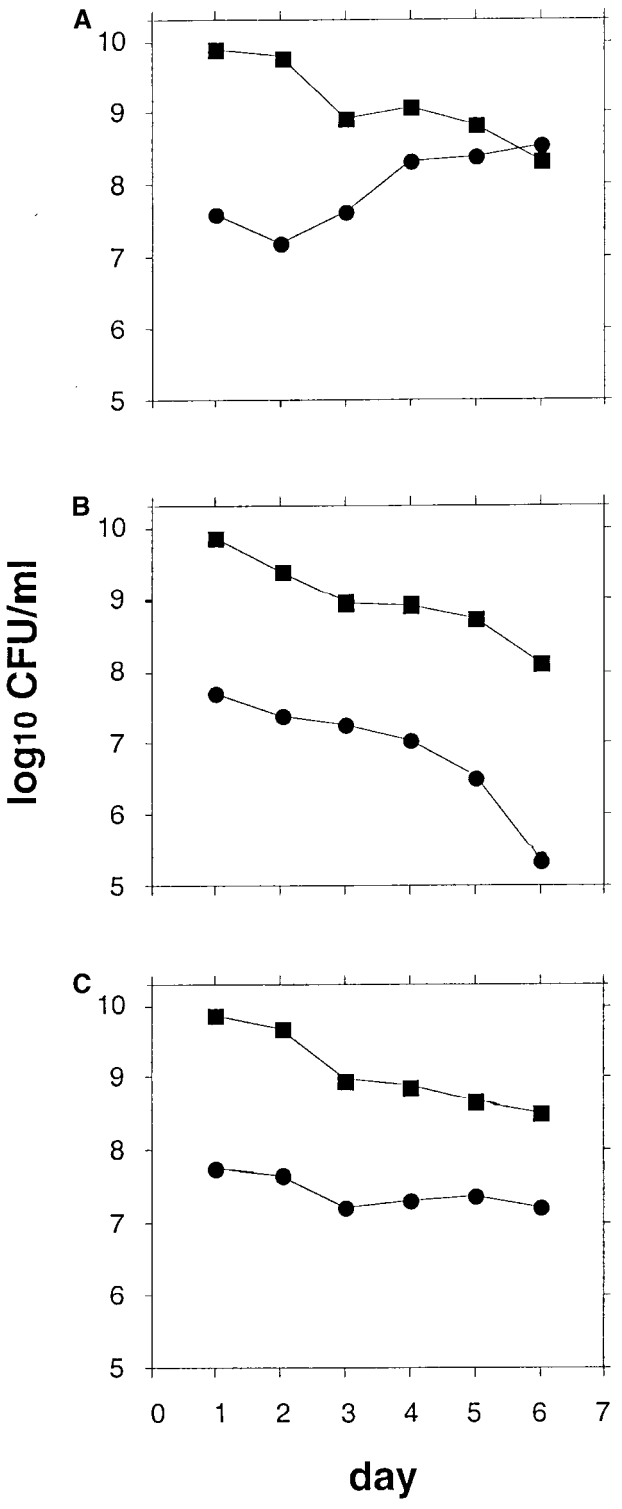

FIGURE 1.—Mixed-culture experiments. One-day-old cultures of both competitors were mixed at a 1:150 ratio. (A) A GASP minority (Bgl^+) mixed with wild-type majority (Val^r); (B) a GASP minority (Bgl^+) mixed with differently marked GASP majority (Val^r); and (C) a wild-type minority (Bgl^+) mixed with differently marked wild-type majority (Val^r).

The survival parameter we compared in each case was the net rate of change in viable counts of both competitors between days 3 and 5. This particular time frame was chosen as representative for two reasons: the GASP phenotype is expressed only after enough nutrients have been released by cells undergoing stochastic death, which in LB happens typically by day 2 (Figure 1A), and it is early enough in the competition that the effect of independent GASP mutants that arise, in either the wild type or the GASP population itself, is minimal.

To determine if the change in viability counts of strains in a mixed population is dependent upon the initial frequency of one of the competitors (ratio of its CFU counts to the total), we calculated the mean rate of change in viable counts of both competitors at different initial frequencies of minority competitor. Figure 2 shows the relationship between these mean survivorship rates for GASP and wild-type strains and their initial frequencies in different competitions (log transformed to perform linear regression analyses).

There is a clear frequency dependence of the survivorship parameter for both competitors in the case of GASP/wild-type competition (Figure 2A). This is not due to a marker effect, as shown by the absence of such frequency dependence when the same parameter for the GASP strain is measured in competition with the same GASP strain carrying another marker (Figure 2B). The same is true in the competition between wild-type strains with the same combination of markers (Figure 2C). Therefore, the survival advantage of the GASP mutant, reflected in its overall increase in viable counts, is largest when it is rare in the wild-type population and decreases as it becomes more abundant. On the other hand, the net death rate of the wild type increases as GASP strain frequency increases, meaning that the presence and growth of the GASP strain negatively affects survival of the wild type. Such a result is expected if the strategy of the GASP mutants is defection from the cooperating wild type. Even though there is a negative effect of frequency on its survival advantage, the GASP strain is expected to reach fixation eventually because at any given initial frequency its survivorship rate is greater than that of the wild type at the corresponding frequencies.

To determine directly whether conditioned medium indeed contains nutrients that both wild type and the GASP strain can utilize for survival and/or growth, we inoculated them into 3-day-old spent medium and followed the change in viable counts over next 2 days (Figure 3). Both wild type and GASP mutants can grow when inoculated at low density in medium conditioned by the wild type, which proves the presence of nutrients beneficial to both genotypes. Another prediction concerning cooperative behavior of the wild type is that

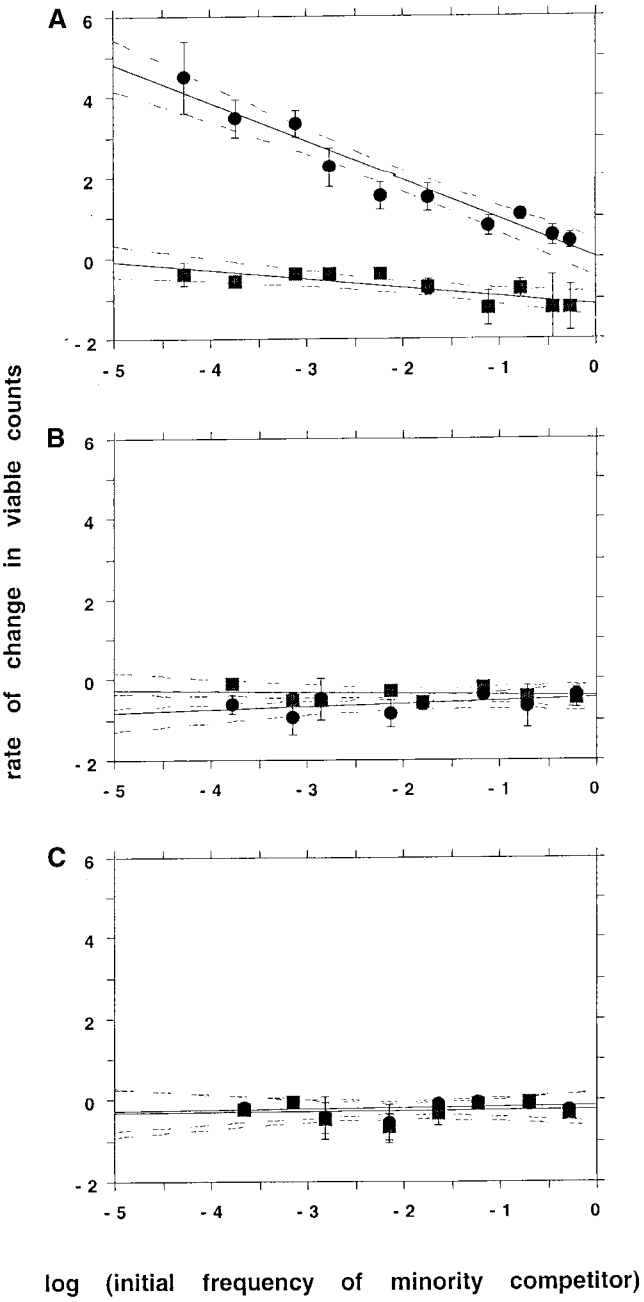

FIGURE 2.—The rate of change in viable counts ("survivorship rate") of a strain expressing GASP phenotype is dependent on its initial frequency in competition. (A) Rate of change in viable counts of GASP strain (●, Bgl^+) and wild-type (■, Val^r) strain in mixed culture (linear regression analysis: GASP slope = −0.962, r = 0.971, t = −11.436, $P < 0.0001$; wild-type slope = −0.219, r = 0.829, t = −4.203, P = 0.0030); (B) rate of change in viable counts of GASP strain (●, Bgl^+) and differently marked (■, Val^r) GASP strain (linear regression analysis: GASP/Bgl^+ slope = −0.029, r = 0.205, t = 1.263, P = 0.2533; GASP/Val^r slope = 0.074, r = 0.458, t = −0.512, P = 0.6271); (C) rate of change in viable counts of wild-type strain (●, Bgl^+) and differently marked (■, Val^r) wild-type strain (linear regression analysis: wild-type/Bgl^+ slope = 0.022, r = 0.134, t = 0.335, P = 0.7487; wild-type/Val^r slope = 0.018, r = 0.095, t = 0.235, P = 0.8220). Dashed lines indicate the 95% confidence interval.

A

	0 h	48 h
WT	$7.60\times10^5 \pm 1.82\times10^5$	$4.67\times10^7 \pm 1.55\times10^7$
GASP (*rpoS819*)	$1.04\times10^6 \pm 2.44\times10^5$	$7.90\times10^7 \pm 3.34\times10^7$

B

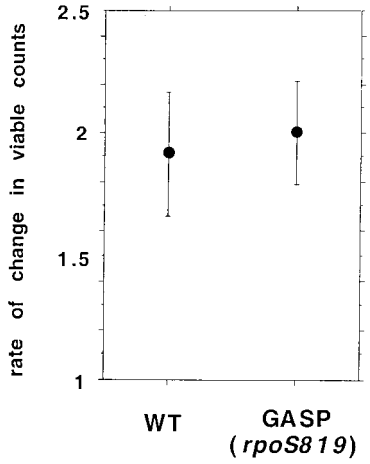

FIGURE 3.—The rate of change in viable counts of wild-type and GASP strains in medium conditioned by the wild type. (A) Mean viable counts (CFUs per milliliter ± SE) of wild type and GASP mutant at the time of inoculation and after 48 hr. (B) The rate of change in viable counts was calculated by applying the same transformation as in Figure 2 to the data from A. Indicated values are the mean of four independent measurements; error bars indicate 1 SE.

it should stop dividing before the GASP strain as the nutrients are becoming exhausted at the end of the growth phase. Indeed, the GASP mutant attains higher cell density than wild type when growing in pure culture in fresh LB medium (Figure 4), which is consistent with this hypothesis.

In that light the evolution of strain(s) exhibiting a GASP phenotype can be described as follows: defection, investing energy in growth by scavenging nutrients still present in the medium and those released by dead cells rather than into maintenance, and stress resistance, confers an important survival advantage over a wild-type strain remaining in a state of nongrowth while nutrients are scarce. The defecting mutant therefore rapidly takes over, adversely affecting the survival of the wild type. In that process the number of cooperators relative to defectors declines. This in turn lowers the defector's survival success, which eventually becomes lower than the initial survival ability of the cooperating ancestor.

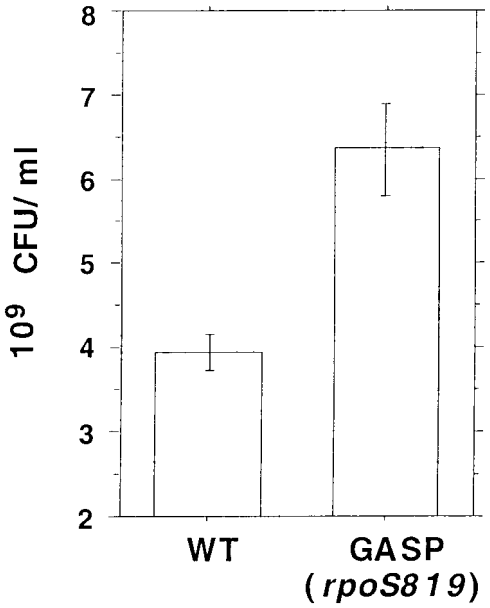

FIGURE 4.—Maximal cell densities reached in LB medium after 12 hr. Indicated values are the mean of three independent measurements (18 cultures per genotype in total); error bars indicate 1 SE.

However, that does not prevent the defector from reaching fixation because the survival ability of the cooperator when it becomes rare is even lower, which means that it does pay off to cheat despite the low payoff.

These dynamics are a result of specific interactions of wild type and the GASP mutant in the condition that promotes growth arrest in the wild type. Evolutionary game theory provides a framework for analyzing cooperation/defection conflicts so we used the survivorship parameter we measured for both competitors to try to construct a payoff matrix typical of a defection/cooperation strategy contest. However, such a matrix concerns interactions between pairs of players, not large groups as is the case in our experiments. The survivorship rate we measured is the average for the entire population of a given competitor, which is a result of the cumulative payoff each member of that population engaged in simultaneous interactions receives. Therefore, we extrapolate our experimentally measured parameters to the value that would be obtained for a single cell of given competitor, in which case its payoff is a result of one type of interaction only.

We used the data obtained to estimate the values $(1 - s_1)$, $(1 + s_2)$, and $(1 - c)$. The survivorship rate of the GASP strain $(1 + s_2)$ is maximal as its frequency approaches 0 in the wild-type population. In that case the survivorship rate of the wild-type strain is 1, and therefore one can estimate the value of $(1 + s_2)$ by comparing the survivorship rates of both competitors extrapolated to an initial frequency of a single GASP strain cell in a wild-type population, using the regression lines in Figure 2A. However, such an extrapolated value for the GASP strain would be several orders of magnitude greater than the closest experimental datum, which would compromise the accuracy of the estimate. This notwithstanding, it is clear from the data that $(1 + s_2)$ is much larger than 1, reflecting a large survival advantage (temptation) for the GASP strain, which is growing and multiplying exponentially while the wild-type strain is dying. At frequencies of the GASP strain approaching fixation the survivorship rate of the wild type reaches its lowest value. Thus, by comparing wild-type survivorship rates extrapolated to a minority competitor's strain frequency of 1 in the case of wild-type/wild-type (Figure 2B) and GASP/wild-type (Figure 2A) competitions, respectively, we get an estimate of $(1 - s_1)$. The latter value is -1.145, and because the survival of the wild type is independent of the initial frequency of another wild-type competitor, the former value equals the average survivorship rate of the wild type at all initial frequencies in wild-type/wild-type competitions (-0.215 ± 0.058 SE, $n = 48$); hence $(1 - s_1) = 0.1878$, reflecting the decrease in survivorship rate of the wild type in the presence of a GASP mutant. The survivorship rate of the GASP strain relative to that of wild type when they are in GASP- and wild-type-only competitions (-0.428 ± 0.058 SE, $n = 62$; -0.215 ± 0.058 SE, $n = 48$), respectively, is 0.5023. That is an estimate of $(1 - c)$, reflecting the decrease in survivorship rate of GASP strains in pure culture compared to the wild type. The ordering of the survival parameters we measured is as follows: temptation $(1 + s_2) \gg 1 >$ reward $= 1 >$ punishment $(1 - c) = 0.5023 >$ sucker's payoff $(1 - s_1) = 0.1878$ and therefore might be interpreted as a case of prisoner's dilemma.

The prisoner's dilemma has been extensively used to explain different biological phenomena (AXELROD and HAMILTON 1981; MILINSKI 1987; TURNER and CHAO 1999). However, due to the inherent difficulty of measuring fitness, only rarely have attempts to specify and measure payoff values succeeded in clearly demonstrating it. The relative simplicity of our experimental system allows us to measure the payoff with enough precision to establish a possible example of the prisoner's dilemma. This example would extend the application of the prisoner's dilemma to prokaryotes, emphasizing the generality of this model in describing biological interactions in diverse organisms.

Our experiments show that mutants that readily take over stationary-phase cultures of *E. coli* can be described as cheaters. Because of the large initial survival advantage they experience, they should arise in any sufficiently large nondividing population. *E. coli* mutants selected under different starvation conditions seem to be of the same type (ROZEN and LENSKI 2000; VASI and LENSKI 1999). Multiple successive takeovers have been observed in long-term starved cultures under laboratory conditions. Typically, early takeovers are complete whereas later ones are not, resulting in the coexistence

of multiple mutant forms (Finkel and Kolter 1999). This dynamic can result from the change in the relative payoff values over time. Early takeovers could conform to the prisoner's dilemma and later ones to another game theory setup known as "chicken game" (Sigmund 1995). In the latter case the sucker's payoff is greater than the punishment, preventing a defector from taking over, resulting in a mixed population. Allelic variation found in the *rpoS* gene in strains isolated from long-term laboratory cultures (Gupta 1997; Sutton *et al.* 2000) as well as from host organisms (Waterman and Small 1996) and secondary environments (Gupta 1997) of *E. coli* (soil and water) suggests that such population takeovers may be occurring in nature.

The question arises as to how a "tight," nonattenuated σ^s can be retained if it is so vulnerable to invasion by its attenuated counterparts. The answer might involve the factors influencing "temptation," the typical amount of time between two "feast" periods, the evolutionary tradeoff associated with attenuation of σ^s, and the spatial structure of the population. The large value of temptation we measured is typical of our experimental condition, rich LB medium, in which the mortality rate of wild type is relatively high and population shifts are hence very rapid. In other conditions, especially minimal media, the mortality of wild type is low, takeovers are consequently slower, and expected temptation is lower, which is probably more similar to natural *E. coli* environments (Siegele *et al.* 1992). The time needed for a complete takeover by attenuated σ^s mutants relative to the time period between two feast periods will obviously influence the potential for a takeover to occur. In a low-nutrient stressful environment such as soil and water, maintenance and stress resistance functions are of major importance for long-term survival, functions most probably compromised in cheating mutants having an attenuated starvation σ-factor. Another point at which they could be counterselected is upon reentering the digestive tract of mammals, the primary environment of *E. coli*. To survive exposure to the low pH encountered in the stomach, *rpoS* function(s) are important (Small *et al.* 1994; Price *et al.* 2000). To test this we measured the survival of GASP and wild-type strains from mixed cultures following low pH stress. Whereas wild type was barely affected, CFU counts of the GASP strain dropped >10,000-fold (Figure 5), clearly showing an evolutionary tradeoff resulting from the attenuation of σ^s.

Waterman and Small (1996) found attenuated *rpoS* alleles in 11 out of 58 clinical isolates as opposed to 10 out of 11 environmental isolates found by Gupta (1997). Those findings may reflect the fact that the cheating mutants that easily arise and take over stationary-phase populations dominate secondary environments whereas they are outcompeted upon reentry into their primary environment. Another parameter that has the potential for limiting cheating is a population's spatial organization. Models show that interaction limited

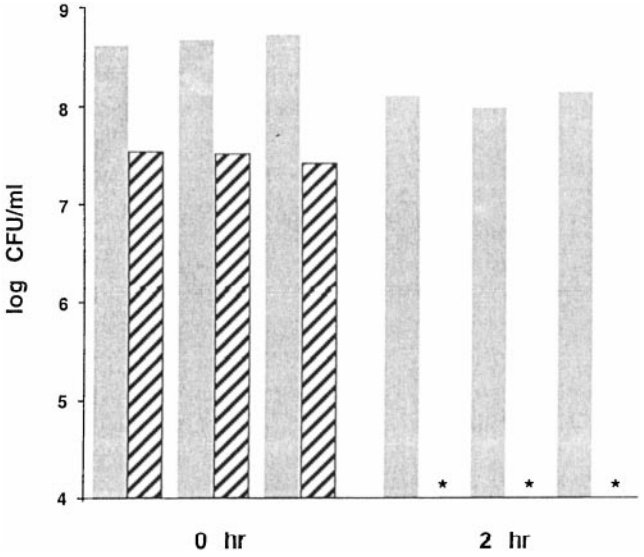

Figure 5.—Survival of wild-type and GASP strains from mixed culture following acid stress. Shown are CFU counts of wild-type (shaded bars) and GASP (hatched bars) strains from three independent cultures before and after exposure to pH 2.5 in fresh medium. An asterisk represents CFU counts $<10^4$/ml.

to immediate neighbors in a structured environment with a local "winner" gaining site (territory) in each generation gives rise to spatially chaotic patterns in which cooperation and defection persist indefinitely (Nowak and May 1992). *E. coli* readily forms biofilms, which are an example of such a structured environment (Pratt and Kolter 1998), but very little is known regarding the degree of structure in natural *E. coli* populations. Finally, there is a question concerning the multispecies communities in which bacteria are usually found in nature. Interspecific competition would always push toward total exhaustion of nutrients; therefore, saving them for maintenance after growth cessation would be very difficult to attain. However, it is not excluded that all species in a typical multispecies community employ the same strategy, in which case it would be a case of interspecific cooperation. Even though no universal quorum-sensing signal molecule has been described so far, such a possibility is not ruled out. The presence of homologues of certain genes involved in quorum sensing across different taxa lends support to such a possibility (Surette *et al.* 1999). Another possibility is that *E. coli* during starvation has very few or no interactions with other (noncooperating) species. Clearly, the evolutionary significance of cheaters cannot be assessed without detailed knowledge of the ecology and population structure of a given lineage.

On the basis of our results showing that cheating underlies population shifts in stationary-phase cultures of *E. coli*, we would predict that in any other case of a growth-arrested clonal population (*e.g.*, other bacteria or unicellular eukaryotes) cheaters that resume growth

would readily arise, unless mutations conferring the cheater phenotype are difficult or impossible to obtain. It could also happen in populations of nondividing cells in multicellular organisms, in which case the cheaters resuming growth would manifest as cancerous cells (Tomlinson 1997). In each particular case the cheaters' fate would be defined by the specific evolutionary tradeoffs associated with the mutations they carry and the spatial structure of the population. Those tradeoffs and spatial organization themselves would be defined by the nature of the environments a given cheater is expected to experience during its typical life cycle.

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LITERATURE CITED

Axelrod, R., and W. D. Hamilton, 1981 The evolution of cooperation. Science **211:** 1390–1396.

Baca-DeLancey, R. R., M. M. T. South, X. Ding and P. N. Rather, 1999 *Escherichia coli* genes regulated by cell-to-cell signaling. Proc. Natl. Acad. Sci. USA **96:** 4610–4614.

Finkel, S. E., and R. Kolter, 1999 Evolution of microbial diversity during prolonged starvation. Proc. Natl. Acad. Sci. USA **96:** 4023–4027.

Finkel, S. E., E. Zinser, S. Gupta and R. Kolter, 1997 Life and death in stationary phase, pp. 3–16 in *Life and Death in Stationary Phase*, edited by S. J. W. Busby, C. M. Thomas and N. L. Brown. Springer-Verlag, Berlin.

Goodrich-Blair, H., and R. Kolter, 2000 Homocysteine thiolactone is a positive effector of sigma(S) levels in *Escherichia coli*. FEMS Microbiol. Lett. **185:** 117–121.

Gorden, J., and P. L. C. Small, 1993 Acid resistance in enteric bacteria. Infect. Immun. **61:** 364–367.

Gupta, S., 1997 *Mutations That Confer a Competitive Advantage During Starvation*. Harvard University, Cambridge, MA.

Harshey, R., and T. Matsuyuma, 1994 Dimorphic transition in *Escherichia coli* and *Salmonella typhimurium*: surface-induced differentiation into hyperflagellate swarmer cells. Proc. Natl. Acad. Sci. USA **91:** 8631–8635.

Hengge-Aronis, R., 2000 The general stress response in *Escherichia coli*, pp. 161–178 in *Bacterial Stress Responses*, edited by G. Storz and R. Hengge-Aronis. ASM Press, Washington, DC.

Huisman, G., and R. Kolter, 1994 Sensing starvation: a homoserine lactone-dependent signaling pathway in *Escherichia coli*. Science **265:** 537–539.

Huisman, G. W., D. A. Siegele, M. M. Zambrano and R. Kolter, 1996 Morphological and physiological changes during stationary phase, pp. 1672–1682 in *Escherichia coli and Salmonella*, edited by F. C. Neidhardt, R. Curtiss, C. A. Gross, J. L. Ingraham, E. C. C. Lin *et al.* American Society for Microbiology, Washington, DC.

Lazazzera, B. A., 2000 Quorum sensing and starvation: signals for entry into stationary phase. Curr. Opin. Microbiol. **3:** 177–182.

Liu, X., C. Ng and T. Ferenci, 2000 Global adaptations resulting from high population densities in *Escherichia coli* cultures. J. Bacteriol. **182:** 4158–4164.

Loewen, P. C., B. Hu, J. Strutinsky and R. Sparling, 1998 Regulation in the *rpoS* regulon of *Escherichia coli*. Can. J. Microbiol. **44:** 707–717.

Maynard-Smith, J., 1982 *Evolution and the Theory of Games*. Cambridge University Press, Cambridge, MA.

Milinski, M., 1987 TIT FOR TAT in sticklebacks and the evolution of cooperation. Nature **325:** 433–435.

Notley, L., and T. Ferenci, 1996 Induction of RpoS-dependent functions in glucose-limited continous culture: what level of nutrient limitation induces stationary phase of *Escherichia coli*? J. Bacteriol. **178:** 1465–1468.

Nowak, M. A., and R. M. May, 1992 Evolutionary games and spatial chaos. Nature **359:** 826–829.

Pratt, L. A., and R. Kolter, 1998 Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Mol. Microbiol. **30:** 285–293.

Price, S. B., C. M. Cheng, C. W. Kaspar, J. C. Wright, F. J. DeGraves *et al.*, 2000 Role of *rpoS* in acid resistance and fecal shedding of *Escherichia coli* O157:H7. Appl. Environ. Microbiol. **66:** 632–637.

Rozen, D. E., and R. E. Lenski, 2000 Long-term experimental evolution in *Escherichia coli*. VIII. Dynamics of a balanced polymorphism. Am. Nat. **155:** 24–35.

Shapiro, J. A., and M. Dworkin, 1997 *Bacteria as Multicellular Organisms*. Oxford University Press, New York/Oxford.

Shimkets, L. J., 1999 Intercellular signaling during fruiting-body development of *Myxococcus xanthus*. Annu. Rev. Microbiol. **53:** 525–549.

Siegele, D. A., M. Almirón and R. Kolter, 1992 Approaches to the study of survival and death in stationary phase *Escherichia coli*, pp. 151–169 in *Starvation in Bacteria*, edited by S. Kjelleberg. Plenum, New York.

Sigmund, K., 1995 *Games of Life*. Penguin, London.

Small, P., D. Blankenhorn, D. Welty, E. Zinser and J. L. Slonczewski, 1994 Acid and base resistance in *Escherichia coli* and *Shigella flexneri*: role of *rpoS* and growth pH. J. Bacteriol. **176:** 1729–1737.

Surette, M., M. Miller and B. Bassler, 1999 Quorum-sensing in *Escherichia coli*, *Salmonella typhimurium* and *Vibrio harveyi*: a new familiy of genes responsible for autoinducer production. Proc. Natl. Acad. Sci. USA **96:** 1639–1644.

Sutton, A., R. Buencamino and A. Eisenstark, 2000 *rpoS* mutants in archival cultures of *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **182:** 4375–4379.

Tomlinson, I. P. M., 1997 Game-theory models of interactions between tumour cells. Eur. J. Cancer **33:** 1495–1500.

Turner, P. E., and L. Chao, 1999 Prisoner's dilemma in an RNA virus. Nature **398:** 441–443.

Vasi, F. K., and R. E. Lenski, 1999 Ecological strategies and fitness tradeoffs in *Escherichia coli* mutants adapted to prolonged starvation. J. Genet. **78:** 43–49.

Waterman, S. R., and P. L. Small, 1996 Characterization of the acid resistance phenotype and *rpoS* alleles of shiga-like toxin-producing *Escherichia coli*. Infect. Immun. **64:** 2808–2811.

Zambrano, M. M., D. A. Siegele, M. Almirón, A. Tormo and R. Kolter, 1993 Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures. Science **259:** 1757–1760.

Zinser, E. R., and R. Kolter, 1999 Mutations enhancing amino acid catabolism confer a growth advantage in stationary phase. J. Bacteriol. **181:** 5800–5807.

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