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LONG-TERM EXPERIMENTAL EVOLUTION IN *ESCHERICHIA COLI*. II. CHANGES IN LIFE-HISTORY TRAITS DURING ADAPTATION TO A SEASONAL ENVIRONMENT

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Abstract.—Twelve populations of the bacterium *Escherichia coli* were propagated for 2,000 generations in a seasonal environment, which consisted of alternating periods of feast and famine. The mean fitness of the derived genotypes increased by ~35% relative to their common ancestor, based on competition experiments in the same environment. The bacteria could have adapted, in principle, by decreasing their lag prior to growth upon transfer to fresh medium (L), increasing their maximum growth rate (V_m), reducing the concentration of resource required to support growth at half the maximum rate (K_s), and reducing their death rate after the limiting resource was exhausted (D). We estimated these parameters for the ancestor and then calculated the opportunity for selection on each parameter. The inferred selection gradients for V_m and L were much steeper than for K_s and D. The derived genotypes showed significant improvement in V_m and L but not in K_s or D. Also, the numerical yield in pure culture of the derived genotypes was significantly lower than the yield of the common ancestor, but the average cell size was much larger. The independently derived genotypes are somewhat more variable in these life-history traits than in their relative fitnesses, which indicates that they acquired different genetic adaptations to the seasonal environment. Nonetheless, the evolutionary changes in life-history traits exhibit substantial parallelism among the replicate populations.

For decades, a major focus in evolutionary ecology has been to elucidate the adaptive significance of variation in life histories (Fisher 1930; Cole 1954; Lack 1966; MacArthur and Wilson 1967; Levins 1968; Pianka 1970; Luckinbill 1978, 1984; Charlesworth 1980; Meuller and Ayala 1981; Reznick 1983; Rose 1984, 1991; Caswell 1989; Partridge and Sibly 1991; Stearns 1992). Here, we intend life histories to include not only age-specific patterns of survival and reproduction but also differences in demographic responses to such environmental factors as population density and resource availability. According to all of these various formulations of life-history theory, a key issue is the relative importance of reproductive contributions made at different ages or under different ecological circumstances to the overall growth of a population. In an expanding age-structured population, for example, reproduction late in life is discounted relative to reproduction at an earlier age, since the earlier progeny will themselves begin to reproduce sooner than the later progeny (Fisher 1930; Lenski and Service 1982).

Beyond distinguishing genetic and environmental influences, a fundamental empirical challenge is determining the extent to which observed variation in life-

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history traits actually reflects adaptation to different environments. Alternatives to strictly "adaptationist" explanations for variation in life-history (or any other) traits include the effects of independent genetic ancestries, which may constrain subsequent evolution, as well as the effects of stochastic processes such as random mutation and genetic drift, which can cause divergence even when initially identical populations evolve in identical environments (Wright 1932, 1982; Gould and Lewontin 1979; Wade 1979; Cohan 1984; Barton and Rouhani 1987; Clarke et al. 1988; Lenski 1988; Cohan and Hoffmann 1989; Mani and Clarke 1990; Harvey and Pagel 1991; Lenski et al. 1991).

We are conducting a long-term experimental study of evolutionary adaptation and divergence, using the bacterium *Escherichia coli*. Twelve populations, each founded from the same clone, were serially propagated for 2,000 generations (300 d) in replicate environments that subjected the bacteria to alternating periods of feast and famine (Lenski et al. 1991). We have previously assessed the rate and extent of genetic adaptation to this "seasonal" regime (see Stewart and Levin 1973) by competing the ancestral and derived genotypes in the experimental environment. (The ancestral genotype was stored in a frozen state. Prior to each competition experiment, both genotypes were acclimated physiologically to the experimental environment.) Relative fitness was calculated as the ratio of the realized Malthusian parameters during the competition experiments. After 2,000 generations, the mean fitness of the derived populations relative to their common ancestor had increased by ~35%. However, any divergence in competitive fitness among the replicate populations was quite small (and might be explained simply by stochastic variation in the timing of equivalent favorable mutations in the replicate populations). In other words, the replicate populations were remarkably similar in the extent of their improvement, even though the genetic variation available for selection was derived entirely from new mutations that occurred independently in each population.

In this article, we seek to identify the changes in life-history traits that were responsible for the demonstrable adaptation of these bacterial populations to the seasonal environment. Our measure of relative fitness takes into account the differential survival and reproductive success of the ancestral and derived genotypes over an entire growth cycle consisting of several more or less distinct phases: (1) a lag phase upon transfer to fresh medium, prior to the commencement of cell division; (2) a period of sustained exponential growth, during which the available resource concentration is little affected because of the low population density; (3) a transition period, in which the limiting resource becomes progressively depleted and the rate of population growth is correspondingly diminished; and (4) a stationary phase, in which the lack of resources prevents cell replication but death may occur. The demographic parameters that govern these phases can therefore be regarded as *components* of fitness, whereas fitness itself is integrated over the entire growth cycle (just as the fitness of an organism with a complex life cycle is an integrated function of its age- or stage-specific fitness components). In addition to these fitness components, we measure certain other life-history traits, including cell size and numerical yield in pure culture. We address whether the changes in life-history traits were similar between the replicate populations

or whether the populations achieved similar fitness gains but by quite different underlying changes in their demography. We also examine whether the observed changes in life-history traits correspond to the opportunity for selection on those traits that can be calculated from a simple model of resource-based competition in a seasonal environment.

MATERIAL AND METHODS

Bacterial Strains

The genotypes used in this study have been described previously (Lenski et al. 1991). Briefly, the common ancestor is a strictly asexual strain of *Escherichia coli* B. The ancestor has two forms, Ara^- and Ara^+, which differ from one another by a single mutation and which can be distinguished by their colony color on tetrazolium-arabinose (TA) indicator agar. Six populations of each arabinose marker type were founded from single cells and then propagated in a serial transfer culture regime (see below). Contamination from external sources was excluded on the basis of genetic markers that distinguished the common ancestor from wild bacteria. Cross-contamination was excluded by propagating Ara^- and Ara^+ lines in a strictly alternating sequence and observing no encroachment of genotypes with the inappropriate marker state. At 2,000 generations (300 d), genotypes were chosen at random from each of the 12 populations. The ancestral and derived genotypes are stored indefinitely at -80°C, so that their properties can be directly compared at any time.

In this study, we used a single clone (genotype) from each of the 12 populations. Previous work has shown that there is little within-population variation for fitness (Lenski et al. 1991), consistent with the expectation of a purging effect caused by selection in an asexual population. Our interest here is to evaluate the parallelism versus divergence of demographic parameters and other life-history traits for independently derived lineages rather than formally to partition genetic variation into its within- and between-population components.

Culture Conditions

Unless otherwise noted, the culture conditions used to measure life-history traits in this study are the same as those used during the long-term evolution of the replicate populations (Lenski et al. 1991). Briefly, this standard environment consists of a glucose-limited minimal salts medium (25 μg glucose mL^{-1}) maintained in a shaking incubator at 37°C. Each day, the populations were diluted 100-fold into fresh medium; they then grew until they had exhausted the available resources (within less than 10 h, as compared to the 24-h transfer interval). The 100-fold daily growth corresponds to ~6.6 ($\log_2 100$) generations of binary fission. To ensure that genotypes were comparably acclimated to the experimental regime prior to assays of competitive fitness or any of the life-history traits, we cultured bacteria for 1 d in a rich broth after removing them from the freezer, and then they were conditioned for 1 d (one complete cycle of lag, exponential growth, transition, and stationary phases) in the standard glucose-limited medium.

Assays of Relative Fitness

The fitnesses of the derived genotypes relative to their common ancestor were assayed in competition experiments under the standard culture conditions, using methods described previously (Lenski et al. 1991). Briefly, in each competition experiment, a derived genotype was competed against the ancestral genotype of the opposite marker state. The Ara marker itself is selectively neutral within $\pm 1\%$ under the standard culture conditions. Following separate conditioning in the standard culture medium, the two competitors were each diluted 1:200 into fresh medium and allowed to compete for 1 d; the competition experiment therefore encompassed the complete cycle of lag, exponential growth, transition, and stationary phases. Initial and final densities of each competitor were estimated by plating onto TA agar. The Malthusian parameter for each competitor is given by

$$m = \log_e(N_f/N_0),$$

where N_0 and N_f are initial and final densities, respectively, and m is therefore a time average (geometric mean) over the entire growth cycle. The fitness, W, of a derived genotype relative to the common ancestor is expressed as the ratio of their Malthusian parameters during a competition experiment. (Lenski et al. [1991] describe the relationship between relative fitness, which is dimensionless, and the selection rate constant, which has units of inverse time.) Five estimates of W were obtained for each derived genotype in sets of complete blocks. These same formulae were also used to calculate relative fitnesses in numerical simulations (see below).

Estimation of Demographic Parameters and Life-History Traits

Measurements of population density.—Estimation of the various demographic parameters required measurements of population density over time. Depending on the particular experiment, we obtained population estimates from viable cell counts based on colony-forming units, from spectrophotometric measurements of optical density, or from particle counts using a Coulter electronic particle counter (model ZM and channelyzer model 256). At fairly high cell densities, spectrophotometric measurements are the easiest to obtain, while particle counts are probably the most accurate method. However, neither spectrophotometry nor particle counts are effective either at very low density or in distinguishing between viable and nonviable cells, when plate counts become necessary.

Maximal growth rate.—The maximal growth rate, V_m, for each genotype was estimated under the standard culture conditions, except that the medium contained 1,000 μg glucose mL^{-1}. (This high glucose concentration is used for estimating V_m in order to be very close to the asymptotic maximal growth rate that is assumed by the Monod model.) After the bacteria were conditioned, they were diluted 1:100 into fresh medium, and the population densities were sampled approximately every half-hour by measuring the absorbance (proportional to density) of the culture with a spectrophotometer. The maximal growth rate was estimated by regressing $\log_e$-transformed absorbance against time during expo-

nential growth phase. (Absorbance depends on the size of cells as well as their number; however, this dependence does not affect the estimate of V_m provided that cell size remains constant during exponential growth. We will show later that cell size differs between exponential growth and stationary phases; however, cell size appeared to be constant within the period of exponential growth used to estimate V_m.) Seven estimates of V_m were obtained for each genotype (including both marker states for the ancestor), in sets of complete blocks.

Resource concentration supporting growth at half the maximum rate.—The glucose concentration, K_s, that supports growth at half the maximum rate provides a measure of a genotype's affinity for glucose as this limiting resource becomes depleted during the transition phase. Preliminary experiments established 0.1 μg glucose mL^{-1} as a concentration at which the genotypes used in this study grew at roughly $V_m/2$, which thus provided a suitable concentration for accurately estimating K_s. After the bacteria were conditioned at this concentration, they were diluted 1:100 into fresh medium, and the population densities were sampled approximately every half-hour by spreading diluted cultures on TA agar. Densities were $\log_e$ transformed and a submaximal rate of increase, V, was estimated for each genotype as the slope of the regression line during exponential growth phase. Using the V_m for each genotype (as estimated above), K_s was then calculated from the model of Monod (1942, 1949):

$$V = V_m S/(S + K_s),$$

so that

$$K_s = S(V_m/V - 1),$$

where S is the resource concentration (here, 0.1 μg glucose mL^{-1}). For each genotype (including both marker states for the ancestor), we obtained two estimates of K_s in sets of complete blocks.

Duration of lag phase.—Each genotype was grown under standard culture conditions, and densities were estimated approximately every hour, using an electronic particle counter. Cultures were diluted 100-fold into an isotonic solution, and the particles in 0.05 mL of the resulting dilution were counted and sized. The frequency distributions for particle sizes exhibited a distinct trough between background particles and bacterial cells. The distributions were edited to eliminate the background counts, which yielded densities that agreed well with those based on viable plate counts. The duration of lag phase, L, was estimated by subtraction, as follows. First, for each growth curve, we measured the initial population density, N_0. Second, we assumed that a population was near the midpoint of exponential-phase growth by the time that its density was $\sim 10N_0$. The population sizes sampled on either side of $10N_0$ were designated N_1 and N_2, respectively, and the corresponding sample times at which they were observed, t_1 and t_2. Third, using the V_m and K_s for each genotype (as estimated above), we inferred the hypothetical times T_1 and T_2 required to reach densities N_1 and N_2, respectively, *if* there was no lag phase prior to exponential growth. That is,

$$T_i = \log_e(N_i/N_0)/V,$$

where $V = V_m S/(S + K_s)$. Finally, the duration of lag phase was then estimated as the average difference between hypothetical and actual times to reach the densities on either side of $10N_0$:

$$L = (t_1 - T_1 + t_2 - T_2)/2 .$$

We obtained three estimates of L for each genotype (including both marker states for the ancestor), in sets of complete blocks.

Death rate during stationary phase.—Death rates during stationary phase, D, were estimated for each genotype under standard culture conditions. Death rates were obtained over the period of 11–24 h after transfer into fresh medium. By 11 h, all genotypes had entered stationary phase, as indicated by both direct observation of population densities and computation of expected dynamics using the previously estimated growth parameters. Densities of viable cells were measured by spreading diluted samples onto TA plates approximately every 2 h during this period. The natural logarithm of cell density was regressed against time during stationary phase, and the regression coefficient provides an estimate of the corresponding death rate. Three independent estimates of D were obtained for each genotype (including both marker states for the ancestor), in sets of complete blocks.

Death rate during prolonged starvation.—During the 2,000 generations of evolution of the experimental populations, cells were transferred into fresh medium every 24 h, and so death rates over longer periods of starvation, D', were not directly subject to natural selection. Nonetheless, these death rates might have changed as a correlated response to selection on some other traits, and so we also assayed death rates over the period of 1–14 d (24–336 h) after transfer into fresh medium. Densities of viable cells were measured by spreading diluted samples onto TA plates at least every other day during this period. The natural logarithm of cell density was regressed against time during prolonged starvation, and the regression coefficient provides an estimate of the corresponding death rate. Four independent estimates of D' were obtained for each genotype (including both marker states for the ancestor), in sets of complete blocks.

Numerical yield in pure culture.—We define the numerical yield, Y, of a genotype as the number of viable cells per unit of limiting resource, when that genotype is grown in isolation (i.e., in the absence of any competitor). The reciprocal of yield therefore provides a measure of the efficiency of conversion of the limiting resource into cell numbers during population growth. Yields in the standard culture medium were estimated at 24 h after transfer into fresh medium by viable cell counts on TA agar plates, divided by the resource concentration (25 μg glucose mL^{-1}). In principle, one could adjust the yield obtained at the end of the growth cycle for cell death that occurred during stationary phase. In fact, however, there was no discernible cell death during this period (see Results), and so no adjustment was made. Five independent estimates of Y were obtained for each genotype (including both marker states for the ancestor), in sets of complete blocks.

Average cell size.—Cell sizes were measured electronically (see above), which indicated the volume displaced by a particle rather than its mass. Size distribu-

tions were edited to remove background particles, which were generally distinctly smaller than the cells (see above). The particle sizer was calibrated using the modal size of highly uniform Coulter latex microspheres with 0.82-μm diameter (0.29 fL, where 1 fL = 10^{-12} mL). Three independent estimates of the average cell size at the end of stationary phase, Z_s, were obtained for each genotype (including both marker states for the ancestor), in sets of complete blocks. We also obtained three independent estimates of the average cell size during the middle of exponential-phase growth, Z_e, for each genotype (including both marker states for the ancestor), in sets of complete blocks.

Statistical Considerations

In evaluating whether the derived genotypes had, as a group, changed relative to their common ancestor, the 12 independently derived lineages were the appropriate unit of replication. In this context, the repeated measures obtained for each genotype were averaged, and they served only to improve the realized precision of our measurements. (We also averaged measurements across the two marker-state variants of the common ancestor.) Except for relative fitness, we employed two-tailed *t*-tests for comparing one value (the average of the two marker states in the common ancestor) with the mean of many values (for the 12 independently derived genotypes), which provides $n_1 + n_2 - 2 = 11$ df (Sokal and Rohlf 1981, pp. 229–231). For relative fitness, we simply compared the mean for the derived genotypes with the value of 1.0 (by definition, the relative fitness of the ancestor), which also provides $n - 1 = 11$ df.

In evaluating whether the derived lineages had diverged significantly from one another, the replicate measures were essential for estimating the relevant error variance. Analyses of variance were performed to test for the significance of between-genotype variation, Var_G, which was estimated as the difference in the group and error mean squares, divided by the number of replicate assays (= blocks) performed per group (Sokal and Rohlf 1981, p. 217). Of course, the common ancestor was not included in these ANOVAs, because the question concerns the divergence of the derived lineages.

Model of Bacterial Population Dynamics

The model used in this study is one of resource-based population growth in a seasonal environment (Stewart and Levin 1973), which has been modified to take into account lag phase prior to exponential growth and to allow cell death during stationary phase. Let the density of bacteria be denoted by N (cells mL^{-1}) and the concentration of the limiting resource by S (μg glucose mL^{-1}). For numerical analysis, we integrate the dynamics in three parts. The first part consists of lag phase, during which time we assume that a population neither grows nor consumes resources:

$$dN/dt = dS/dt = 0.$$

The second part consists of both exponential and transitional growth phases, during which time the population increases and resources are consumed at rates depending on the parameters of the Monod model:

$$dN/dt = [V_m S/(S + K_s)]N,$$

and

$$dS/dt = -(1/Y)(dN/dt),$$

where V_m (h^{-1}), K_s (μg glucose mL^{-1}), and Y (cells μg^{-1} glucose) are the maximal growth rate, the resource concentration at which growth rate is half of the maximum, and the numerical yield, respectively. The third part consists of stationary phase, during which time the cell population may decline owing to death, while the resource concentration remains constant:

$$dN/dt = -DN$$

and

$$dS/dt = 0,$$

where D (h^{-1}) is the death rate.

The first part runs from $t = 0$ to $t = L$, where L(h) is the duration of lag phase and t is the time after transfer into fresh medium. The second part then runs until $S = 0$, that is, until the resource has been exhausted. The third part runs until $t = 24$ h, at which time the cycle is completed. If two genotypes are competing, they may enter the second part at distinct times, depending on the duration of their lag phases. Because competing genotypes share the same pool of limiting resource, they necessarily enter the third part simultaneously.

In our numerical analyses, we sought to duplicate the conditions of our experiments. We therefore began with a medium containing 25 μg glucose mL^{-1}, then added to this 1/100th of a corresponding volume of a stationary-phase cell culture. Thus, the initial resource concentration and population density were

$$S(0) = 0.99(25)$$

and

$$N(0) = 0.01\ Y(25).$$

In the case of two competing genotypes, each one has an initial density of only $0.005\ Y(25)$.

Equations were integrated numerically using SOLVER.SWV, which uses the fourth-order Runge-Kutta method of integration and allows discrete switch points in dynamic models (Blythe et al. 1990). We used time steps of 0.0005–0.01 h in the numerical integrations, depending on the precision required for solution of a particular problem. The criterion for the end of the second part, $S = 0$, is approached asymptotically in a truly continuous model, so that one must begin the third part when the resource concentration reaches some arbitrarily low threshold criterion. In a simulation involving two competing genotypes, their relative fitness over the entire cycle is expressed using the same formula used to estimate relative fitness empirically (see above).

TABLE 1

ANCESTRAL FITNESS COMPONENTS AND PROPORTIONAL SELECTION GRADIENTS

Fitness Component	Ancestral Value, X	Selection Gradient, $(X/W)(\partial W/\partial X)$	Maximum Fitness
L	1.5264 h	−.2554	1.3051 (L = 0)
V_m	.7726 h^{-1}	1	∞ ($V_m = \infty$)
K_s	.0727 $\mu g\ mL^{-1}$	−.0066	1.0069 (K_s = 0)
D*	(0) h^{-1}	0	1 (D = 0)

* The actual estimate of D was negative, but it was not significantly different from zero. The population dynamic model does not allow negative death rates (i.e., growth after the glucose has been exhausted), and so D has been set to zero in these analyses.

RESULTS

Ancestral Traits and Opportunity for Selection

Table 1 gives estimates of the growth parameters for the common ancestor. The maximal growth rate, V_m, corresponds to a doubling time ($\log_e 2/V_m$) of a little less than 1 h. The duration of lag phase prior to growth, L, is well over 1 h. Also, K_s is very low relative to the initial resource concentration (25 μg glucose mL^{-1}); in fact, the ancestral genotype is predicted to grow at half of its maximum rate even when 99.7% of the glucose has been used. The estimated death rate during stationary phase, D, is slightly negative, which suggests the possibility of continued growth at a slow rate (and hence some deficiency in the model of population dynamics). However, this trend is not statistically significant. (It is also possible that some cell death occurred but was offset by continued cell division. However, the methods we employed do not allow this distinction to be made.) Figure 1 shows a numerical simulation of the dynamics of the ancestral genotype and the limiting resource over the course of one 24-h cycle, assuming neither growth nor death during stationary phase.

Some, but not all, published estimates of K_s for *Escherichia coli* strains grown in minimal media with glucose as the sole carbon source are more than an order of magnitude higher than our own estimate (Monod 1949; Luckinbill 1984; but see Shehata and Marr 1971 for an estimate within a few percentage points of our estimate). Therefore, we were concerned with the possibility that our method of estimating K_s might somehow be biased. One possible bias is that we might have measured cell division without concomitant cell growth, which may occur transiently after transfer into fresh medium. This explanation would imply that the ancestral genotype could not indefinitely sustain population growth consistent with our estimate of K_s. A second possible bias is that we might have measured growth on organic contaminants of the medium or glassware or on citrate that is included in the medium (which some bacteria other than *E. coli* can use as a carbon source) rather than on glucose. This explanation would imply that our ancestral genotype could grow at a similar rate in a medium with less or even no added glucose. However, we excluded both of these possible artifacts by showing

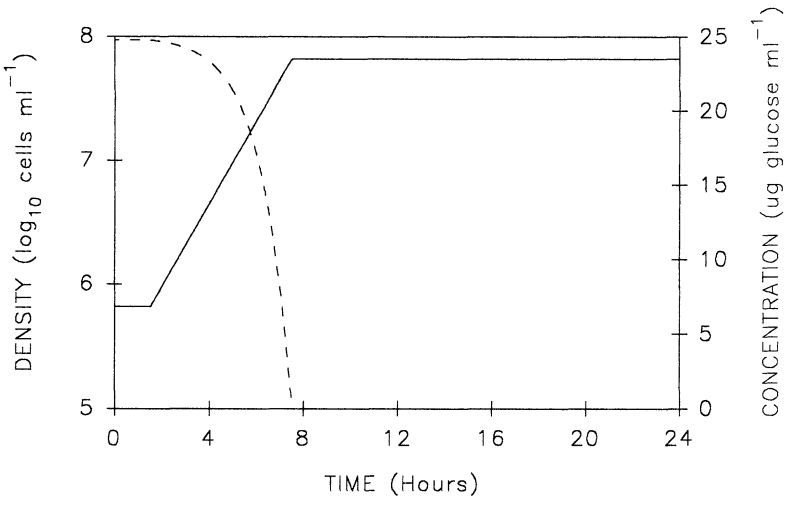

FIG. 1.—Numerical integration of the dynamics of a bacterial population (*left axis, solid line*) and the limiting resource (*right axis, dashed line*), using the parameters estimated for the common ancestor (table 1) and the standard culture conditions.

that our bacteria can indefinitely sustain a 100-fold daily increase at glucose concentrations 0.04 μg mL^{-1} and higher, but not at concentrations of 0.02 μg mL^{-1} and below. The 100-fold daily increase implies $V > \log_e 100/24$ h $= 0.19$ h^{-1}. Given that $V = V_m S/(S + K_s)$ and with $V_m = 0.77$ h^{-1}, this rate of increase can be sustained in a medium that contains 0.04 μg glucose mL^{-1} only if $K_s < 0.12$ μg glucose mL^{-1}. By the same logic, the failure of the bacteria to persist in a medium containing 0.02 μg glucose mL^{-1} implies that $K_s > 0.06$ μg glucose mL^{-1}. If K_s were less than this value, then the bacteria should have persisted even at this low concentration. We conclude that our estimate of K_s (table 1) is quite accurate.

One can formalize the opportunity for selection acting directly on each fitness component as follows. We begin with a genotype having the growth parameters estimated for the common ancestor (except death rate during stationary phase, which is set to zero, since the model does not allow for continued growth after the limiting resource has been exhausted). We then introduce a second genotype that is identical except for a very small change in one parameter. Next, we simulate competition between these two genotypes over an entire growth cycle and compute the fitness of the improved genotype relative to the ancestor, as described in Material and Methods. The selection gradient for any trait X is defined by the partial derivative of fitness with respect to that trait (cf. Lande 1982), $\partial W/\partial X$, which we have obtained by the limit of $\Delta W/\Delta X$ as ΔX goes to zero. This gradient therefore reflects the direct selection acting on each fitness component, with the other components held constant. (In effect, this method ignores any interaction between the demographic parameters by assuming that each one can be changed independently of the others.) To facilitate comparisons among the

selection gradients for the several fitness components (which have different units), we have scaled these gradients to reflect the proportional sensitivity of fitness to each component, rendering them all dimensionless quantities:

$$G_X = (X/W)(\partial W/\partial X),$$

where $W = 1$ for the ancestor (by definition). (One can also obtain the proportional selection gradients for L and V_m analytically; D. E. Dykhuizen, personal communication.) The proportional selection gradients are shown in table 1.

These proportional selection gradients are equivalent to "elasticities" in population projection matrices (de Kroon et al. 1986) and to "control coefficients" in biochemical models of metabolic flux (Kacser and Burns 1979; Dykhuizen et al. 1987). It is frequently the case in such analyses that the sum of the proportional sensitivities is equal to one (de Kroon et al. 1986; Dykhuizen et al. 1987; Mesterton-Gibbons 1993); however, that is not always true, and it is demonstrably *not* the case for the proportional selection gradients considered here (table 1). The failure of the proportional sensitivities to sum to one implies that W is not a homogeneous function of the fitness components (Mesterton-Gibbons 1993); this inhomogeneity may be a consequence of the nonequilibrium nature of the population dynamics in the seasonal environment.

The proportional selection gradient for the maximum growth rate, V_m, is about four times steeper than the corresponding gradient for the duration of lag, L, and more than 100 times steeper than the gradient for K_s, which governs the reduction in growth rate as the resource becomes depleted. The death rate, D, cannot be reduced below zero according to the model, and so its proportional selection gradient is zero.

The selection gradient for numerical yield, Y, is also zero. This result may seem puzzling at first glance, but in fact it is straightforward: any increase in the efficiency of conversion of resources into cell numbers that does *not* simultaneously increase growth rate cannot enable one genotype to outcompete another, because such a change would (in a physically unstructured environment) concomitantly leave more resources for use by the competitor. One might reformulate the model by replacing V_m in the equation of population growth with the product of a maximum rate of resource uptake ($U_m = V_m/Y$) and yield (Y), such that selection would act on both U_m and Y. We have not done so because we did not measure U_m.

In addition to these proportional selection gradients, we can calculate the theoretical maximum improvement in fitness that could be achieved by changing any one of the parameters from the ancestral state (table 1). There is no theoretical limit to V_m or to the fitness that can be achieved by increasing V_m. But L, K_s, and D cannot be less than zero. Hence, there is a maximum improvement in fitness that can be achieved by changing any of these parameters, with the greatest room for improvement in L (~30%), only slight room for improvement in K_s (<1%), and no room for improvement in D.

Of course, average cell sizes (Z_s and Z_e) and the death rate during prolonged starvation (D') do not enter directly into the dynamic model, and hence selection gradients are not applicable, unless specific couplings between fitness compo-

TABLE 2

CHANGES IN FITNESS COMPONENTS AND OTHER LIFE-HISTORY TRAITS DURING 2,000 GENERATIONS OF EVOLUTION IN A SEASONAL ENVIRONMENT

Life-History Trait	Mean for Common Ancestor	Mean for Derived Genotypes	t_s	P
Relative fitness:				
W	(1)	1.3486	12.450	***
Fitness components:				
L (h)	1.5264	1.2470	−2.458	*
V_m (h^{-1})	.7726	.8887	5.504	***
K_s ($\mu g\ mL^{-1}$)	.0727	.0880	2.215	*
D (h^{-1})	−.0127	.0029	1.243	NS[a]
Other life-history traits:				
Y ($\times 10^6\ \mu g^{-1}$)	2.6352	1.8386	−5.137	***
D' (h^{-1})	.0216	.0156	−2.745	*
Z_s (fL)	.3546	.6549	4.412	**
Z_e (fL)	.5678	1.1357	4.704	***

NOTE.—Except for relative fitness, the null hypothesis is that the means are equal for the common ancestor and the derived genotypes. For relative fitness, the null hypothesis is that the mean fitness of the derived genotypes is one. Significance is based on the two-tailed probability of rejecting the null hypothesis using the t distribution with 11 df.

[a] $.05 < P$.

* $.01 < P < .05$.

** $.001 < P < .01$.

*** $P < .001$.

nents and these traits are assumed. Also, these selection gradients do not take into account possible trade-offs or other couplings between the demographic parameters that do enter into the model. However, one can readily see that selection might favor an allele that improved one of the parameters subject to strong selection (e.g., V_m), even if it compromised one of the other parameters subject to weaker selection (e.g., K_s).

Changes in Life-History Traits

Table 2 compares the average values of the life-history traits for the ancestral and derived genotypes. The derived genotypes improved, on the average, by 35% in fitness relative to the common ancestor. Contributing to this improvement were a ~15% increase in the maximal growth rate (V_m) and a ~20% shorter lag phase (L). The resource concentration at which growth rate was half of the maximum (K_s) was ~20% higher in the derived genotypes, which has a negative impact on fitness. The death rate during stationary phase (D) did not change significantly. Thus, the observed adaptations were in the two demographic parameters with the greatest opportunity for selection.

The numerical yield (Y) of the derived genotypes was reduced by ~30% relative to the common ancestor. That is, whereas the derived genotypes increase in abundance relative to the common ancestor during competition (as indicated by their higher relative fitness), the derived genotypes yield *fewer* cells per unit

resource when they are grown in isolation. At least two distinct hypotheses could account for this result: the derived genotypes may produce larger cells, so that the total biovolume is not reduced; or in the course of their more rapid growth, the derived genotypes may burn the glucose less efficiently or produce more metabolites that inhibit growth, so that the total biovolume is also reduced. Table 2 shows that the derived genotypes were, in fact, ~85% larger in average cell volume in stationary phase (Z_s). Thus, the total biovolume produced over the entire growth cycle (expressed as the product of Y and Z_s) had, in fact, actually increased by ~30% in the derived genotypes. The difference between the derived and ancestral genotypes in cell size during exponential growth, Z_e, was even greater (~100%) than the difference during stationary phase.

Although the death rate during stationary phase (D) did not change significantly, the derived genotypes had a ~30% lower death rate during prolonged starvation (D').

Parallelism versus Divergence in Life-History Traits

Table 3 summarizes the ANOVAs to test for variation among the 12 independently derived genotypes in each of the fitness components and other life-history traits. There was no significant heterogeneity among the derived genotypes in their fitnesses relative to the common ancestor (W).

The derived genotypes also showed no significant genetic variation in their maximal growth rates (V_m), which contributed to their fitness improvement, or in the resource concentrations that allow half of the maximum growth rate (K_s), which were uniformly worse than that of their ancestor. There was, however, significant heterogeneity among the derived genotypes in the duration of lag phase (L), which improved on the average relative to the ancestor, and in death rates during stationary phase (D), which did not change on the average from the ancestral state.

Among the other life-history traits, numerical yield (Y), death rate during prolonged starvation (D'), and average cell sizes (Z_s and Z_e) all exhibited significant heterogeneity among the independently derived genotypes.

Sufficiency of the Demographic Model

Are the demonstrable improvements in maximal growth rate (V_m) and duration of lag phase (L) sufficient to explain the ~35% gain in fitness relative to the common ancestor? Using our demographic model, we simulated competition between a genotype with the ancestral parameters and each of 12 genotypes with parameters corresponding to the independently derived types. Overall, the mean fitness of the derived competitors relative to their common ancestor, using the model and the corresponding parameter estimates, was 1.21, compared with the mean of 1.35 obtained from the actual competition experiments. For all 12 derived genotypes, the simulated fitness was less than the actual fitness, which is highly unlikely by chance ($P = .5^{12} < .001$).

One potentially artifactual explanation for this effect is that all the simulations use the same parameters for the common ancestor, and so all share some errors in estimation that might produce concordance in the deviations between simulated

TABLE 3

GENETIC VARIATION IN FITNESS COMPONENTS AND OTHER LIFE-HISTORY TRAITS AMONG THE 12 INDEPENDENTLY DERIVED GENOTYPES

	GROUP		BLOCK		ERROR			
LIFE-HISTORY TRAIT	MS	df	MS	df	MS	df	Var_G	P
Relative fitness:								
W	7.63×10^{-3}	11	5.77×10^{-3}	4	6.27×10^{-3}	44	2.72×10^{-4}	NS[a]
Fitness components:								
L (h)	3.58×10^{-2}	11	2.05×10^{0}	2	1.54×10^{-2}	22	6.79×10^{-3}	*
V_m (h^{-1})	2.88×10^{-3}	11	1.47×10^{-2}	6	2.55×10^{-3}	66	4.67×10^{-5}	NS[a]
K_s ($\mu g\ mL^{-1}$)	9.58×10^{-5}	11	1.82×10^{-4}	1	1.08×10^{-4}	11	(0)	NS[a]
D (h^{-1})	4.33×10^{-4}	11	8.10×10^{-4}	2	1.81×10^{-4}	22	8.40×10^{-5}	*
Other life-history traits:								
Y (μg^{-1})	1.11×10^{11}	11	4.20×10^{11}	4	4.46×10^{10}	44	1.33×10^{10}	*
D' (h^{-1})	1.75×10^{-5}	11	2.34×10^{-6}	3	1.17×10^{-6}	33	4.09×10^{-6}	***
Z_s (fL)	1.28×10^{-2}	11	3.40×10^{-3}	2	6.12×10^{-4}	22	4.07×10^{-3}	***
Z_e (fL)	4.04×10^{-2}	11	1.23×10^{-2}	2	4.64×10^{-3}	22	1.19×10^{-2}	***

NOTE.—MS and df indicate mean squares and degrees of freedom, respectively. The between-genotype variance component, Var_G, is estimated as $(MS_{group} - MS_{error})/n$, where n is the number of replicate assays (= blocks) per group. Estimates of Var_G may be negative, but the true Var_G must be zero or positive. Level of significance for rejecting the null hypothesis that $Var_G = 0$ is based on the F-test, with degrees of freedom corresponding to the group and error mean squares.

[a] $.05 < P$.

* $.01 < P < .05$.

*** $P < .001$.

and actual competitions. This explanation is not tenable, however, because the uncertainties in the parameter estimates for the ancestral genotype are too small to account for the deviations between simulated and actual fitnesses. To see this, recall that the fitness components were independently estimated for the two marker-state variants of the common ancestor (Ara^- and Ara^+). The simulated relative fitness for these two ancestral genotypes differs by only ~0.01 from the measured relative fitness (data not shown), whereas the simulations between the derived and ancestral genotypes differ by more than 0.09 in all 12 cases. Evidently, the discrepancy between the relative fitnesses predicted by the estimated parameters in the simple model of competition and the observed fitnesses is real, with the model accounting for only ~60% [(1.21 − 1)/(1.35 − 1)] of the improvement. In the Discussion, we consider possible explanations for the remaining 40% of the fitness gain.

Genetic Correlations between Traits

We calculated the product-moment correlations between the estimated values of the eight fitness components and other life-history traits for the 12 derived genotypes. Only two of the 28 pairwise correlations are individually significant, and only one of these (between Z_s and Z_e) is significant at $P < .05$ after correcting for multiple tests using the sequential Bonferroni criterion (Rice 1989). However, these genetic correlations between extant populations do not necessarily tell us very much about the correlated responses that occurred within populations in the past.

DISCUSSION

We have been investigating the changes that occur during long-term evolution in a simple experimental system. Twelve bacterial populations, founded from the same ancestral genotype, were propagated for 2,000 generations in replicate environments, which subjected them to alternating periods of feast and famine. The derived genotypes increased in their competitive fitness in this seasonal environment by ~35%, on the average, relative to their common ancestor (Lenski et al. 1991). There was very little divergence among the derived genotypes in their competitive fitnesses relative to one another, despite their dramatic gains relative to their common ancestor and even though their improvements depended entirely on new mutations that occurred independently in the replicate populations (Lenski et al. 1991). However, the populations may have attained similar fitnesses, but by different underlying changes in their life histories.

Therefore, in this study, we sought to determine which demographic parameters were responsible for the improved competitive fitness of the derived genotypes relative to their common ancestor and whether similar life-history changes had occurred among the independently evolving populations. We also addressed whether the observed changes corresponded to those expected from the relative opportunities for selection acting on the various parameters in a simple mathematical model of resource-based population growth in a seasonal environment. In addition, we examined whether the observed changes were sufficient, within the

context of this model, to account for the improvements in fitness that were measured by the competition experiments.

Changes in Fitness Components and Other Life-History Traits during Adaptation to a Seasonal Environment

We believe it is important to distinguish two types of life-history traits. First, there are fitness components per se, which are those demographic parameters that directly determine rates of change in the relative abundance of competing genotypes. If one has sufficient knowledge of these demographic parameters, then one should be able to predict the outcome of competition between genotypes, provided that one's demographic model is sufficiently realistic to encompass the relevant dynamics. In age-structured models of life-history evolution, age-specific survival and fecundity schedules provide these fitness components (Service and Lenski 1982). In models based on Lotka-Volterra dynamics, the fitness components are subsumed by exponential growth rates, carrying capacities, and competition coefficients (Pianka 1970; Gill 1972). In this article, fitness components are represented by the terms defining population dynamics in a resource-based model of competition in a seasonal environment: the duration of the lag (L), the maximum growth rate (V_m), the resource concentration at which the growth rate is half of the maximum (K_s), and the death rate during stationary phase (D). Fitness itself is some function of these parameters, but it need not be equally sensitive to all of them. Moreover, the effect of each parameter on fitness will depend on features of the environment (including the initial resource concentration, the dilution factor, and the frequency of transfers into fresh medium) as well as on the values of the other parameters.

Second, one may believe that other traits underlie fitness (based on intuition, empirical data, or other knowledge of the organism) but lack any theoretical basis to predict the outcome of competition. Traits such as body size or running speed, for example, may correlate with, and even predict empirically, survival and reproductive success. But these traits do not enter into any population dynamic model, except to the extent that they may be used to predict one or more fitness components that do (Arnold 1983).

Populations of *Escherichia coli* that were maintained for 2,000 generations in a seasonal environment showed systematic changes in several fitness components as well as other traits that are presumably physiologically relevant to these components. In particular, the derived genotypes adapted by increasing their maximum growth rates (V_m) and by reducing their lags prior to growth upon renewal of a previously exhausted resource (L).

The derived genotypes also had increased the resource concentration necessary to sustain growth at half of their maximum rate (K_s), seemingly a maladaptive response. However, growth rate asymptotically approaches $V_m S/K_s$ as S goes to zero, so that K_s becomes more important at low resource concentrations, but V_m does not become any less important. In fact, the average ratio, V_m/K_s, for the derived genotypes is very close to that ratio for the ancestral genotype. Thus, the derived genotypes are not actually less fit in competition for very sparse glucose, but they are better adapted to exploiting abundant glucose.

The derived genotypes also showed no improvement in their death rate during stationary phase (11–24 h); if anything, they are worse than their progenitor in this respect. However, the derived genotypes do have improved survival during prolonged starvation (1–14 d). But this trait cannot be considered a fitness component per se, since it is manifest only in environments different from the environment in which the populations evolved. (Had they evolved in an environment in which transfers were made only every 14 d, then this would properly be a fitness component and would enter into a dynamic model of competition in that environment.)

The derived genotypes also had much lower numerical yields (Y) than their common ancestor. Yield enters into the dynamic model of competition, but it does not directly affect fitness in a mass-action environment. However, in a structured environment, in which the probability of extinction of a local deme or the number of dispersing propagules may be functions of population size, numerical yield could well affect the outcome of competition. Our results therefore suggest a possible conflict between intrademic selection, favoring demographic traits that correlate with a reduction in numerical yield, and interdemic selection, favoring higher yield (cf. Wade 1979, 1980). This reduction in numerical yield is evidently caused by a change in the size of the bacterial cells, which became systematically larger in the derived genotypes, rather than by less efficient conversion of resource into biomass. In fact, the total biovolume, expressed as the product of numerical yield and average cell size at stationary phase (YZ_s), was greater for the derived genotypes than for their common ancestor.

Our preconception had been that evolving cells would become smaller (and more numerous), in order to increase the ratio of surface area to volume and hence the effective rate of uptake of the limiting resource. But clearly this preconception was wrong. We can imagine at least four (post hoc) explanations for the evolution of larger cell size. First, survival during stationary phase may have been improved for larger cells. The derived genotypes, which had larger cells, survived better during prolonged starvation. However, there was no measurable cell death (or improvement therein) over the length of stationary phase that the cells experienced during their experimental evolution. Hence, this hypothesis cannot account for the evolution of larger cells, even if it does plausibly explain the observed reduction in death rate during prolonged starvation. Second, larger cell size may provide a physiological basis for the shorter duration of lag phase upon transfer to fresh medium. That is, larger cells may be less depleted of metabolic reserves during stationary phase (even if the smaller-celled ancestor does not actually die), which would allow the derived genotypes to respond more quickly upon renewal of the resource. Third, larger cells, by virtue of their greater total surface area, may have a higher rate of resource uptake per cell (rather than per unit volume), which might enable the derived cells to sequester resources that could subsequently be converted into progeny. This explanation is consistent with the difference in average cell size between exponential and stationary phases, which is larger in the derived genotypes than in the common ancestor; this difference may even account for some of the increase in relative fitness (see below). However, Luckinbill (1984) observed a similar evolutionary trend toward

larger cells, even in populations that never experienced stationary phase or resource limitation, that does not support any of the preceding hypotheses. Finally, cell size may have increased simply because of its allometry with growth rate. Several studies with *E. coli* and related bacteria have documented an allometric relationship between growth rate and cell size, in which growth rate is manipulated *phenotypically* by altering the environment (Schaechter et al. 1958; Neidhardt et al. 1990). Our results indicate a *genetic* relationship between growth rate and cell size. This hypothesis suggests experiments to determine whether the observed genetic relationship is simply an extension of the phenotypic relationship or whether the allometry between growth rate and cell size has been genetically altered.

Parallelism versus Divergence in Life-History Traits

Did all 12 independently derived genotypes exhibit the same suite of adaptive changes in their life histories, or did they find alternative solutions to the challenges imposed by the seasonal environment? At first glance, our results seem to imply substantial divergence of the derived genotypes in the life-history bases of their improved fitness. Two of the four fitness components and all four of the other life-history traits show statistically significant heterogeneity (table 3). However, it is equally important to emphasize that the *direction* of evolutionary change was highly parallel for most of the fitness components and other life-history traits. Three of the four fitness components and all four of the other life-history traits showed significant directional trends in their mean values (table 2). In fact, all 12 independently derived genotypes showed the same outcome of higher V_m, higher K_s, lower D', lower Y, higher Z_s, and higher Z_e than their common ancestor. And 11 of the 12 derived genotypes had lower L and higher D than the ancestor. Thus, there was near-perfect uniformity in the directional responses exhibited by the replicate populations.

An index of the relative extent of divergence versus parallelism is given by the following ratio:

$$I_X = \sqrt{\mathrm{Var}_G(X)}/|\Delta X|,$$

where $\mathrm{Var}_G(X)$ is the between-genotype variance for trait X, and ΔX is the average change from the common ancestor. (By using the standard deviation corresponding to the genetic variance, both the numerator and denominator have the same units.) This index therefore provides a measure of the average difference among the independently derived genotypes relative to the average evolutionary change from the ancestral state.

For all eight fitness components and other life-history traits, this index is much less than one (table 4), which indicates that the differences among the independently derived genotypes are small relative to the average change in these characters from the ancestral state. However, the independently derived genotypes are somewhat more variable in most of these traits than in their relative fitnesses (also based on this index), which implies that they have acquired slightly different adaptations to the seasonal environment. Evidently, the 12 replicate populations underwent similar adaptations in terms of the fitness components and other life-

TABLE 4

DIVERGENCE VERSUS PARALLELISM IN FITNESS COMPONENTS AND OTHER LIFE-HISTORY TRAITS FOR THE INDEPENDENTLY DERIVED GENOTYPES

Life-History Trait, X	$\mathrm{Var}_G(X)$	ΔX	$\sqrt{\mathrm{Var}_G(X)}/\lvert\Delta X\rvert$
Relative fitness:			
W	2.72×10^{-4}	.349	.047
Fitness components:			
L (h)	6.79×10^{-3}	−.279	.295
V_m (h^{-1})	4.67×10^{-5}	.116	.059
K_s ($\mu g\ mL^{-1}$)	(0)	.015	0
D (h^{-1})	8.40×10^{-5}	.016	.573
Other life-history traits:			
Y (μg^{-1})	1.33×10^{10}	-7.97×10^{5}	.145
D' (h^{-1})	4.09×10^{-6}	−.006	.337
Z_s (fL)	4.07×10^{-3}	.300	.213
Z_e (fL)	1.19×10^{-2}	.568	.192

NOTE.—$\mathrm{Var}_G(X)$ is the between-genotype variance for trait X (table 3), and ΔX is the mean change from the common ancestor (table 2). The ratio $\sqrt{\mathrm{Var}_G(X)}/\lvert\Delta X\rvert$ provides a measure of the average difference among derived genotypes (divergence) relative to the average change from the ancestral state (parallelism).

history traits, but these were not identical, as indicated by heterogeneity among the independently derived genotypes for some of the characters.

Adequacy of the Dynamic Model Used to Amalgamate Fitness Components into a Measure of Relative Fitness

The population model that we have used might be regarded as explanatory in two distinct ways. First, how well does the model predict the outcome of competition between ancestral and derived genotypes, when the relevant demographic parameters for each genotype are estimated independently of the competition experiments? Second, did the model provide any indication of the most likely evolutionary changes in these parameters?

After 2,000 generations of evolution, the mean fitness of the derived genotypes relative to their common ancestor was ~1.35, whereas the predicted mean fitness based on the model and independently estimated parameters was only ~1.21. In other words, the simple model of resource-based competition in a seasonal environment accounts for only about 60% (0.21/0.35) of the observed adaptation. The discrepancy is not simply due to a lack of statistical resolution, because all 12 derived genotypes gave actual and predicted relative fitnesses that differed in the same direction and by amounts much greater than can be explained by uncertainty in the demographic parameters.

We see two major types of limitation of the simple model that might account for the unexplained portion of the fitness differential between the ancestral and derived genotypes. First, the description of the mechanistic basis for competition may be inadequate. The model used here assumes that competition is mediated entirely by scramble competition for glucose. However, bacteria excrete metabolites that may act as either secondary resources or inhibitors of growth, and these

metabolites may differentially affect competing genotypes (Helling et al. 1987; Levin 1988). If excreted metabolites are involved, then they must work to the advantage of the derived genotypes, because the actual fitness advantages are greater than explained solely on the basis of glucose-mediated competition. However, the fact that population densities were fairly low ($\sim 5 \times 10^5$ cells mL^{-1} to $\sim 5 \times 10^7$ cells mL^{-1} over the course of the growth cycle) in the evolution experiment tends to weaken this explanation, because the concentration of metabolites should be proportional to density. However, one can imagine experiments (employing conditioned media or varying the initial relative frequency of competitors) to test this possibility further.

Second, the description of the bacterial demography may be inadequate to encompass all the relevant dynamics. For example, the model implicitly assumes that the rate at which bacteria increase in numbers is strictly proportional to the rate at which they remove resources from the medium and, furthermore, that these two processes are synchronous. The numerical yield, Y, scales the conversion of resources into bacterial cells during exponential growth. But Y was estimated from stationary-phase populations, which are composed of much smaller cells than exist during the exponential phase (table 2). It seems likely, therefore, that the bacteria are taking up resources during exponential growth faster than the ratio V_m/Y ($\mu g\ h^{-1}$ per cell) would imply. If so, then a more appropriate measure of the maximum growth rate (in terms of accounting for the observed fitness improvement) would be

$$V_{m,adj} = V_m Z_e / Z_s .$$

The product $V_m Z_e$ reflects the rate of increase in biovolume during exponential phase growth, whereas the denominator indicates the eventual (but asynchronous) conversion of this biovolumetric increase into cells of size Z_s. Therefore, $V_{m,adj}$ takes into account the more rapid accumulation of biomass by the bacteria than would be apparent from their numerical increase during the exponential phase. We calculated $V_{m,adj}$ for each genotype and then used these adjusted maximum growth rates (along with the other demographic parameters) to compute the expected fitnesses of the derived genotypes relative to their common ancestor. The mean expected fitness using the adjusted maximum growth rates was 1.36, which was indistinguishable from the mean value of 1.35 measured in the competition experiments ($P > .5$ based on a t-test for paired comparisons). Thus, the differential underestimation of resource accumulation for the ancestral and derived genotypes can plausibly explain the "missing" 40% of the improvement in fitness. We recognize that this adjustment will give an incorrect trajectory for cell numbers during growth phase. Our intention here is not to provide a highly realistic and complex description of population dynamics but rather to suggest a possible explanation for the deviation between relative fitnesses estimated by direct competition experiments and those predicted from the simple demographic model. To develop a more formal model along the lines of this adjustment, one might model population biomass (rather than cell numbers) or otherwise incorporate more complex functional relationships between substrate concentration, growth rate, and numerical yield.

The other application of the demographic model was to infer the relative intensities of selection acting on the several fitness components. According to the model, selection to increase the maximum growth rate (V_m) and to reduce the duration of the lag prior to growth in fresh medium (L) was much stronger than selection to reduce the concentration of resources required to support half of the maximum growth rate (K_s) and to reduce the death rate during stationary phase (D). Indeed, we observed a good correspondence between these theoretical selection gradients and the actual responses to selection: both V_m and L improved substantially during the experimental evolution, whereas neither K_s nor D showed any improvement.

We note also that V_m and L promote exploitation of the resource when it is abundant, whereas K_s and D are relevant only when the resource has been depleted. Therefore, it is fair to say that the bacteria in this study have adapted to the "feast" but not the "famine" aspect of the seasonal regime. But it is also important to emphasize that the opportunity for selection to act on the various demographic parameters (table 1) depends on the initial state of the life history as well as on the environment. Had either K_s or D been much higher (worse) initially, their corresponding selection gradients would have been steeper and there would have been more room for improvement in these fitness components. As it was, however, K_s and D were both already so low for the ancestral genotype that selection to improve them was very weak. The fact that our ancestral bacterium was so well adapted to famine conditions suggests strong selection in the past for the corresponding fitness components (see, e.g., Koch 1971, 1985; Mikkola and Kurland 1992).

Of course, a genetic response depends on not only the intensity of selection but also the availability of genetic variation for that trait and on genetic correlations between traits. Had we not observed significant improvements in either strongly selected fitness component (V_m and L), this hypothetical outcome might have been due to a lack of genetic variation or a trade-off with another fitness component. The fact that there was substantial genetic variation in these fitness components (and no evidence for intractable trade-offs) is presumably because the experimental conditions represent a novel environment for these bacteria, whereas in the ancestral environment one would expect those substitutions that improve fitness to have been largely exhausted by prior selection (Service and Rose 1985; Lenski et al. 1991; Bennett et al. 1992).

In contrast to the results reported here, Dykhuizen and Hartl (1981) observed significant improvements in both V_m and K_s for *Escherichia coli* that evolved in glucose-limited continuous culture (chemostats). This difference in outcomes is not unexpected, however, in light of the difference in experimental environments imposed. In continuous culture, bacteria hold the equilibrium concentration of limiting resource, S^*, to a value that is exactly sufficient to offset washout from the vessel (Stewart and Levin 1973; Hansen and Hubbell 1980). In the study by Dykhuizen and Hartl (1981), the flow rates through the culture vessels, f, were either 20% or 40% of the founding bacterium's maximal growth rate, V_m. Given that $V = V_m S^*/(S^* + K_s) = f$, then S^* would have been either 0.25 K_s or 0.67 K_s, respectively, according to the Monod model. Here V is the actual Malthusian

parameter in continuous culture, and so the proportional selection gradients for V_m and K_s differ by a factor of

$$[(V_m/V)(\partial V/\partial V_m)]/[(K_s/V)|\partial V/\partial K_s|] = (S^* + K_s)/K_s$$
$$= S^*/K_s + 1.$$

Thus, the proportional selection gradients for V_m and K_s in the study by Dykhuizen and Hartl (1981) differed by only a factor of 1.25 or 1.67 (depending on the flow rate), in contrast to the more than 100-fold difference in the corresponding gradients in the seasonal environment used in this study (table 1).

Also using *E. coli* in a glucose-limited medium as a model system, Luckinbill (1978, 1984) employed two serial transfer environments to test for specific adaptations to *r*- and *K*-selection regimes. In the *r*-selection regime, bacteria were kept in a state of perpetual exponential growth by repeated transfers before the populations had reached stationary phase; in the *K*-selection regime, bacteria were allowed to exhaust the glucose and enter stationary phase before they were transferred into fresh medium. Luckinbill demonstrated improvements in relative fitness in both environments, but he found that the adaptations were nonspecific with respect to the selection regime. That is, bacteria that had evolved in the *K*-selection environment did as well, on the average, in the *r*-selection environment as bacteria that had evolved in the *r*-selection environment, and vice versa. But the *K*-selection regime employed by Luckinbill was, in fact, quite similar to the seasonal environment used in this study. And as we have discussed, this seasonal environment selects most strongly for a higher maximal growth rate, with much weaker selection for any traits that might be construed as *K* selected (at least when the ancestral genotype has life-history components similar to the strain used in this study). So Luckinbill's intended *K*-selection regime may, in fact, have been another strongly *r*-selecting regime. The changes in bacterial fitness components reported by Dykhuizen and Hartl (1981) and Luckinbill (1978, 1984), as well as those observed in this study, appear to be consistent with the relative intensities of selection acting on those traits. These analyses indicate to us the utility of an explicit demographic model for interpreting evolutionary changes in life-history traits.

In conclusion, 12 bacterial populations that evolved independently for 2,000 generations in replicate seasonal environments underwent a similar suite of changes in their life histories. The derived genotypes responded more quickly to resource renewal and had higher maximum growth rates than their common ancestor. Both of these adaptations facilitate resource exploitation during the periods of feast in the seasonal environment. But the derived genotypes showed no corresponding improvement in those fitness components that would allow them to better tolerate the periods of famine that they also experienced. The differential adaptation to periods of feast and famine can be understood in terms of the relative opportunities for selection to act on the different fitness components. In particular, the ancestral genotype used in this study was already so well adapted to the periods of resource deprivation imposed by the experimental regime that there was almost no room for improvement in the corresponding fitness compo-

nents, whereas there was substantial room for improvement in the fitness components that mattered during the periods of resource abundance. Accompanying these demographic adaptations was a dramatic change in the morphology of the bacteria, with the derived genotypes having much larger cells than their common ancestor. The significance of this morphological change with respect to their newly evolved life history is unclear.

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