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EVOLUTIONARY ADAPTATION TO TEMPERATURE. IV. ADAPTATION OF *ESCHERICHIA COLI* AT A NICHE BOUNDARY

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Abstract.—Following an environmental change, the course of a population's adaptive evolution may be influenced by environmental factors, such as the degree of marginality of the new environment relative to the organism's potential range, and by genetic factors, including constraints that may have arisen during its past history. Experimental populations of bacteria were used to address these issues in the context of evolutionary adaptation to the thermal environment. Six replicate lines of *Escherichia coli* (20°C group), founded from a common ancestor, were propagated for 2000 generations at 20°C, a novel temperature that is very near the lower thermal limit at which it can maintain a stable population size in a daily serial transfer (100-fold dilution) regime. Four additional groups (32/20, 37/20, 42/20, and 32–42/20°C groups) of six lines, each with 2000 generation selection histories at different temperatures (32, 37, 42, and daily alternation of 32 and 42°C), were moved to the same 20°C environment and propagated in parallel to ascertain whether selection histories influence the adaptive response in this novel environment. Adaptation was measured by improvement in fitness relative to the common ancestor in direct competition experiments conducted at 20°C. All five groups showed improvement in relative fitness in this environment; the mean fitness of the 20°C group after 2000 generations increased by about 8%. Selection history had no discernible effect on the rate or final magnitude of the fitness responses of the four groups with different histories after 2000 generations. The correlated fitness responses of the 20°C group were measured across the entire thermal niche. There were significant tradeoffs in fitness at higher temperatures; for example, at 40°C the average fitness of the 20°C group was reduced by almost 20% relative to the common ancestor. We also observed a downward shift of 1–2°C in both the upper and lower thermal niche limits for the 20°C selected group. These observations are contrasted with previous observations of a markedly greater rate of adaptation to growth near the upper thermal limit (42°C) and a lack of trade-off in fitness at lower temperatures for lines adapted to that high temperature. The evolutionary implications of this asymmetry are discussed.

Key words.—Adaptation, bacteria, correlated responses, *Escherichia coli*, fitness, niche, stress, temperature.

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The interaction between abiotic factors and population genetic processes is important in determining the course of evolutionary change. Temperature fluctuations in particular have been emphasized as a stress factor that can affect the rate and direction of evolutionary change (Parsons 1987; Hoffmann and Parsons 1991; Lenski and Bennett 1993). It has been proposed that selection conditions that border on the limits of an organism's ability to persist may result in rapid evolutionary responses (Hoffmann and Parsons 1991; Bennett et al. 1992; Howarth 1993). In addition, if there is genetic coupling between characteristics that govern performance at the high and low ends of the range for an environmental factor, then adaptation to one extreme may be associated with trade-offs that reduce performance in other environments (Huey and Kingsolver 1989) and could channel the future evolution of the niche in one direction or another (Brooks and McLennan 1991). We have designed an experimental system that allows us to monitor evolutionary adaptation in the bacterium *Escherichia coli* in response to changes in an important environmental factor, temperature, and we evaluate the results in light of such evolutionary predictions.

This particular experiment arose from previous studies that employed natural selection in the laboratory to examine adaptation of *E. coli* populations to different thermal regimes (Bennett et al. 1990, 1992; Bennett and Lenski 1993). In those studies, a single bacterial clone, with an evolutionary history at 37°C, was used to found replicate experimental lines that were propagated in four thermal regimes: 32, 37, and 42°C, and a daily alternation between 32 and 42°C. After 2000 generations, these experimental groups were found to have adapted (as indicated by improved fitness relative to their common ancestor) to their particular selective thermal environments. The groups were not found, however, to have significantly altered their ancestral niche (defined as the range of temperatures over which they could maintain their populations during daily serial dilution culture). The lower boundary of this common thermal niche was found to be approximately 19.5°C. We therefore undertook this experiment in which a new experimental group was founded from the original common ancestor and propagated at 20°C for 2000 generations. This experimental group thus has a parallel evolutionary history to our original four experimental groups and supplements our original set to span the entire ancestral thermal niche. Its creation permits us to address a series of questions about the rate and extent of adaptation at the lower thermal niche edge and its correlated responses. In addition to the creation of the 20°C group, the replicate lines of the four original experimental groups were moved to the 20°C environment and propagated for a further 2000 generations in this novel thermal environment. These groups are designated 32/20, 37/20, 42/20, and 32–42/20°C to indicate their historical thermal exposure. The thermal histories of each of these lines has thus been controlled for 6000 generations; for example, the 32/20°C group had a history of 2,000 generations at 37°C, followed by 2000 generations at 32°C, and now a further 2000 generations at 20°C. A phylogeny, thermal history, and taxonomy of these experimental lineages is presented in Figure 1.

Analysis of these groups permits us to address several

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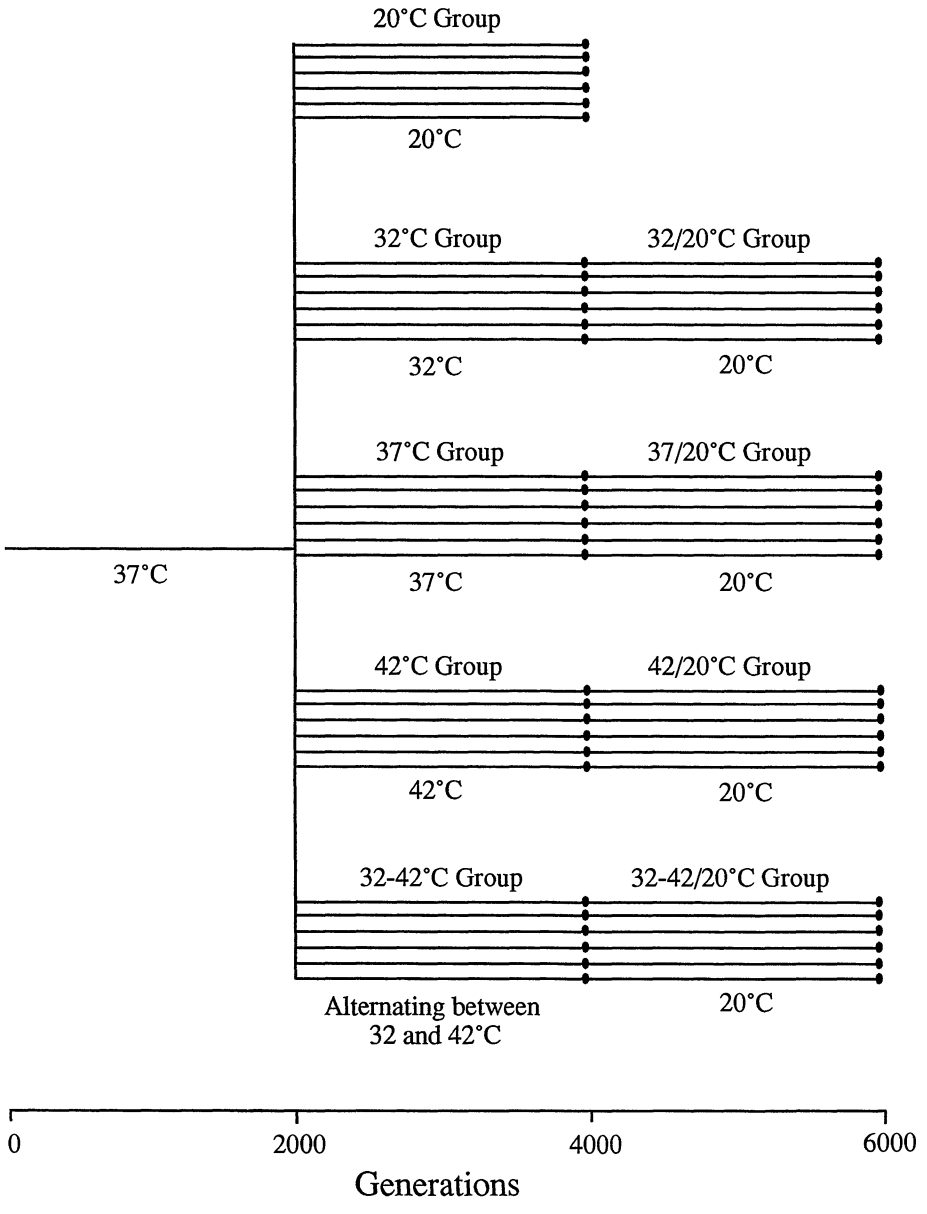

FIG. 1. Phylogeny, thermal history, and taxonomy of experimental lines and groups used in this study. Group names are given above and culture temperatures below the designated lines. The experimental line at 37°C for the first 2000 generations is described in Lenski et al. (1991) and the 32, 37, 42, and 32–42°C groups are derived from Bennett et al. (1992). All other groups derive from this study.

questions that are relevant to adaptive evolution in general and evolutionary responses to temperature in particular. First, do populations adapt more rapidly to temperatures at the extreme limits of their thermal niche? In our previous studies (Bennett et al. 1990, 1992), we observed that the 42°C group adapted much more rapidly and achieved a much greater eventual increase in fitness than the groups maintained at lower, and more moderate, temperatures. We proposed that one explanation for this differential response to natural selection could be the severity of selection at the limits of thermal tolerance. Alternatively, it might reflect some asymmetry between higher and lower temperatures in the stringency of selection. The upper thermal boundary is considerably sharper than the lower, where population growth gradually slows at increasingly colder temperatures until the population can no longer persist. If proximity to the edge of a niche boundary per se results in more rapid evolution, we would expect to see more rapid and pronounced fitness responses to both niche extremes. In this case, adaptation to 20°C should be similar in rapidity and extent to that observed at 42°C, both being greater than adaptation to an intermediate temperature such as 32°C. In addition, this project was intended to explore further the existence of correlated tradeoffs associated with thermal adaptation. Does adaptation to an environmental variable impact the breadth and shape of an organism's niche with respect to that variable? Finally, the design of this experiment allowed us to examine whether evolutionary history had an effect on the rate and/or ultimate

extent of subsequent adaptation to a novel environment. Does, for instance, prior adaptation to a moderately low temperature (32°C) accelerate adaptation at 20°C relative to prior adaptation to a high temperature (42°C)?

Materials and Methods

Bacterial Strains and Media.—The ancestral strain used in this study was a clone isolated from a population of *E. coli* B that had been maintained by serial propagation in glucose-limited medium for 2000 generations at 37°C (Lenski et al. 1991). An isogenic derivative of that clone that can utilize the sugar L(+)arabinose (Ara^+) was obtained by spontaneous mutation. The Ara^+ and Ara^- phenotypes can be distinguished by plating on tetrazolium arabinose (TA) indicator agar. Six replicate lines were initiated from these clones, three of each marker state. The marker allowed us to monitor the cultures for evidence of cross-contamination between populations as well as to compete the evolving lines in mixed culture with the ancestor bearing the opposite marker. The marker itself has been shown to be effectively neutral under the conditions employed in this study. These six lines are designated as the 20°C group (see Fig. 1).

An additional four groups of six populations each were initiated with clonal isolates obtained by Bennett et al. (1992). In that previous experiment, the 24 lines underwent 2000 generations of evolution parallel to that described above but at different temperatures. Clonal isolates from the populations at the end of that study were used to found new populations that were moved to the 20°C incubator and propagated in parallel with the six lines described above. These four groups of six lines are designated as the 32/20, 37/20, 42/20, and 32–42/20°C groups to indicate the temperature at which they were previously exposed (32, 37, 42, and daily alternation between 32 and 42°C, respectively) and their subsequent exposure to 20°C (see Fig. 1).

The culture media used in this study was Davis minimal (DM) supplemented with glucose (25 μg/ml) (Lenski et al. 1991). The common ancestor as well as all clonal isolates obtained in this study are stored for future analysis in a 12% glycerol suspension at −80°C.

Culture Conditions.—All cultures described here were initiated from single clones and propagated by daily transfer of 0.1 ml of each culture into 9.9 ml of fresh liquid media. The cultures were incubated at 20°C unless otherwise stated and shaken at 120 rpm. The temporal and spatial variation in the incubator was within ±0.5°C. Bacterial populations were enumerated by spreading diluted cultures on TA indicator agar plates, which were incubated at 37°C for one day. The plating medium and temperature represents an arbitrary environment in which to enumerate the abundance of a population.

The evolving lines in all five experimental groups were plated on TA agar after 100, 200, 300, 600, and every succeeding 200 generations through a total of 2000 generations. For each line, a single colony was chosen at random. These clonal isolates were regrown in medium containing glycerol and then stored at −80°C. After 300 generations, several of the populations began to show reduced plating efficiency at 37°C. To reduce the possibility of selecting clones from only a subset of the population, clones were isolated from plates incubated for four days at 20°C from that point onward.

Measurements of Relative Fitness.—Relative fitness was measured by direct competition between each evolved line and the ancestral clone with the opposite Ara marker state. Cultures of the populations to be competed were inoculated from freezer stocks into a rich broth (LB) (Miller 1972) and grown overnight at 37°C. These cultures were then diluted and grown in DM for two days at the appropriate temperature to allow the cultures to become comparably acclimated to the environment (e.g., Leroi et al. 1994). After this preconditioning, the 24-h cultures of each pair of competitors were mixed 1:1 and diluted 100-fold into fresh media. Samples of this mixture were taken immediately and again after 24 h of growth, diluted and plated on TA agar to estimate the initial and final densities of each competitor. Relative fitness (W) is defined as the ratio of the number of doublings achieved during the competition by the experimental line being tested and its ancestor (Lenski et al. 1991). This fitness was calculated as follows:

$$W = \frac{\log_2\left(\frac{N_t}{N_0}\right)}{\log_2\left(\frac{A_t}{A_0}\right)}$$

where N and A are the densities of the evolved and ancestral lines, respectively, and the subscripts 0 and t indicate the initial and final time points (i.e., 0- and 24-h samples). A difference in plating efficiency between the competitors will not affect our estimates of relative fitness as long as plating efficiency is the same at each time point.

Measurements of Absolute Fitness.—Absolute fitness was estimated by regressing the natural logarithm of population density immediately prior to serial dilution against time for each of the ancestral and evolved genotypes in pure culture in the same medium in which they had evolved (see above) but at a variety of different temperatures (see Bennett and Lenski 1993). The slope of the regression is equivalent to the genotype's Malthusian parameter. In order to maintain a constant population size under these conditions, the population must grow at least fast enough to compensate for the 100-fold daily dilution. As long as the population is at its maximum density for the given resource level at the beginning of the experiment, the Malthusian parameter cannot be significantly greater than zero. A slope less than zero indicates that the population is growing more slowly than the dilution rate imposed by the environment. A slope of −4.6 (ln 0.01) indicates that the population is neither growing nor dying, but is simply being diluted from the culture. These experiments were carried out in a shaking water bath incubator with temporal fluctuations of ±0.1°C. The cultures were removed from the incubator for only a few minutes to take a sample of each and transfer it to fresh media.

Results

Rate and Extent of Adaptation of the 20°C Group.—The fitness responses observed during 2000 generations of evolution at 20°C are shown in Figure 2 for the six lines of the

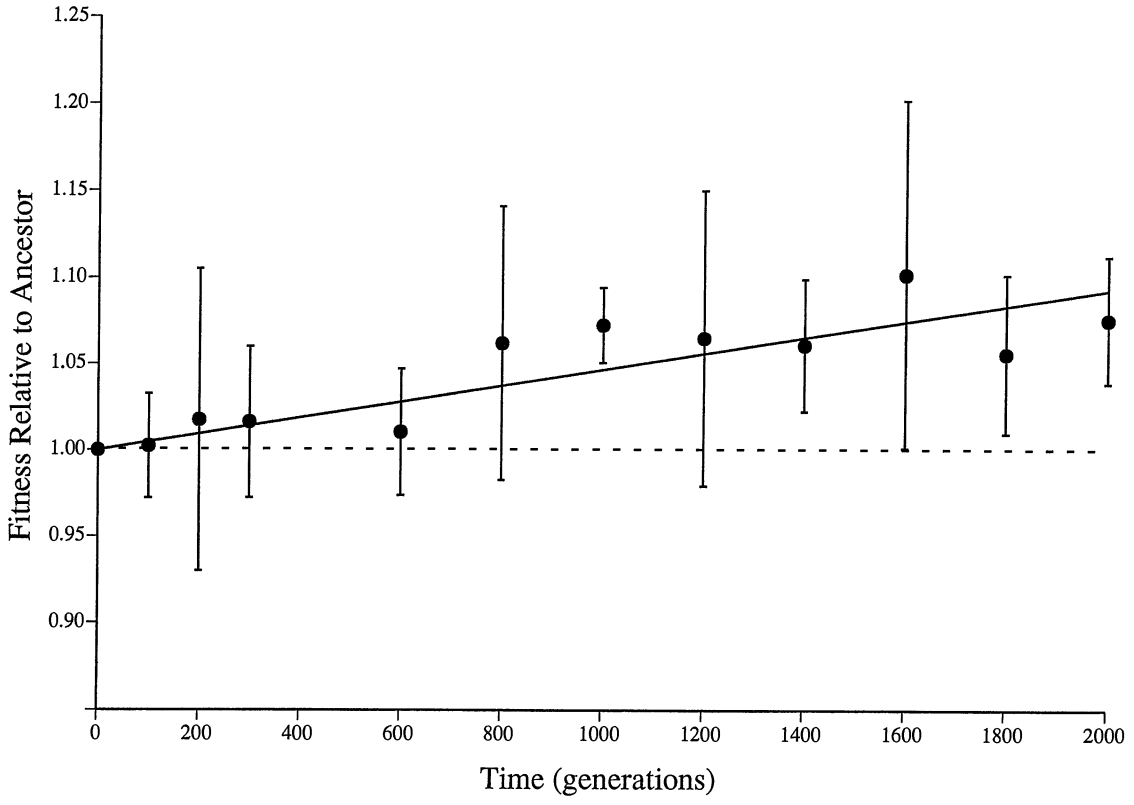

FIG. 2. Fitness response over 2000 generations of evolution at 20°C. Fitnesses of single clones isolated from the evolving populations were estimated relative to the reciprocally marked common ancestor at each time point. The data points are the mean fitness of the six replicate lines at each time point and the error bars represent the 95% confidence intervals. The solid line represents the average linear regression fit to the data points of each of the six lines individually.

TABLE 1. Pairwise comparisons of the direct fitness response among experimental groups obtained over 2000 generations of experimental evolution.

Experimental groups	Mean difference[1]	Mann-Whitney test statistic	Significance[2]	
			I	SB
Comparison of relative fitness at 2000 generations[3]				
20 vs. 32	−0.0195	10	0.240	
20 vs. 37	0.0617	1	0.004	*
20 vs. 42	−0.2480	0	0.002	**
20 vs. 32-42	−0.0867	2	0.009	*
Comparison of rates of adaptation over entire 2000 generations[4]				
20 vs. 32	-1.616×10^{-5}	7	0.078	
20 vs. 37	1.405×10^{-5}	30	0.055	
20 vs. 42	-1.266×10^{-4}	0	0.004	*
20 vs. 32-42	-8.475×10^{-6}	6	0.055	

[1] In every case, the mean difference is calculated by subtracting the value for the second group in the comparison from the value for the 20°C group.

[2] Two-tailed probabilities were calculated for each comparison (*I*) with six lines per group ($n_1 = n_2 = 6$) and by applying the sequential Bonferroni technique to each set of four comparisons (SB). In the latter case, an (*) indicates that the null hypothesis of no difference is rejected at $P < 0.05$ and (**) at $P < 0.01$.

[3] Using data from assays performed by A. F. Bennett and R. E. Lenski (1996).

[4] Using data from Bennett et al. (1992).

20°C group. Mean relative fitness increased during the course of the experiment to a final value at 2000 generations of 1.08 (±0.014 SE). An analysis of variance failed to demonstrate any significant heterogeneity among lines in the magnitude of their relative fitness at the end of the experiment ($F_{5,30} = 1.338$; $P = 0.275$). The mean relative fitness for each line was regressed over time using a linear model to estimate the average rate of change in fitness over the entire 2000 generations; the intercept was constrained to a value of 1.0, which is by definition the ancestral fitness. The average rate of change in relative fitness for the group was 4.658 (±0.104 SE) × 10^{-5} per generation.

One of the primary motivations for this study was to determine whether the fitness response to natural selection at the lower limit of this bacterium's thermal niche would be similar to that observed in response to natural selection at the upper thermal limit (Bennett et al. 1992). To test this hypothesis, we compared both the rate of adaptation and the final mean relative fitness of the 20°C group with published estimates for groups derived from the same ancestral strain in the same environment but at higher temperatures, including the ancestral temperature (37°C) and the upper thermal limit for this bacterium (42°C) (Bennett et al. 1992; Bennett and Lenski 1996). The results are summarized in Table 1. The rates of adaptation and the final fitness for the six replicate lines of the 20°C group were compared with the values for each of the parallel groups by Mann-Whitney tests with prob-

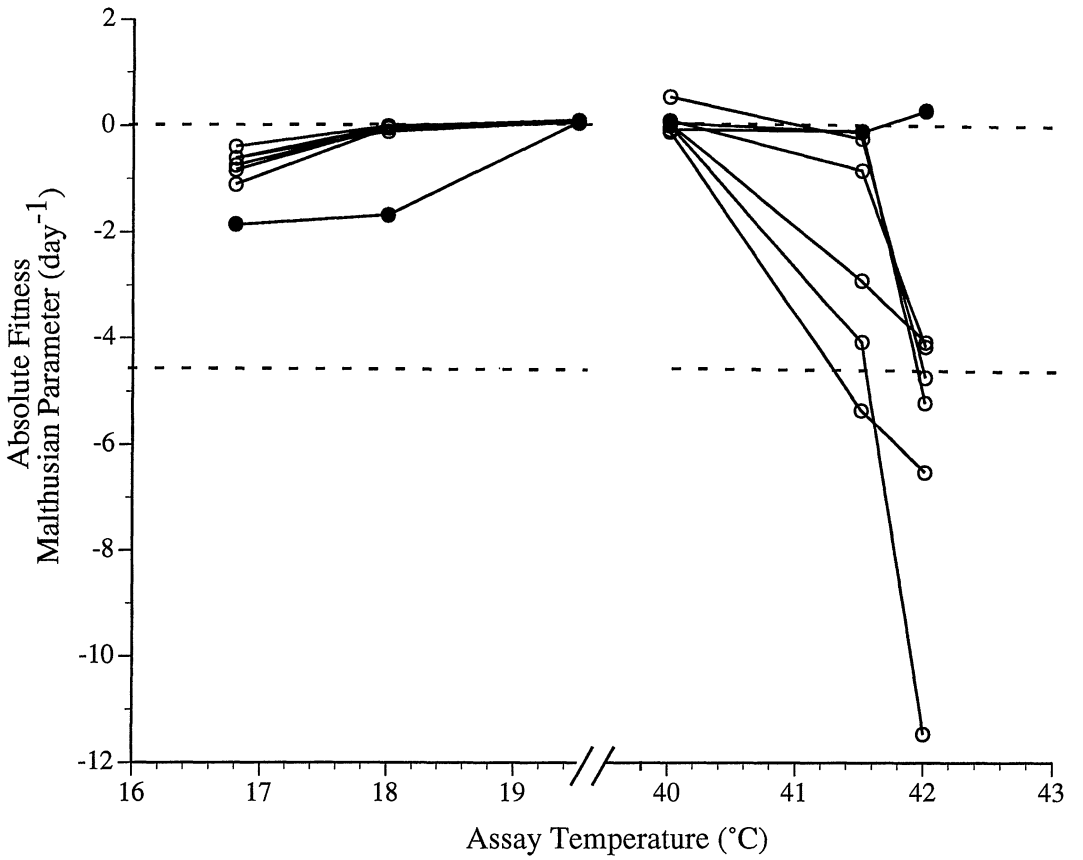

FIG. 3. Mean absolute fitnesses of the ancestor (●) and the six lines of the 20°C group (○) between 17 and 42°C. Means are based on two replicate assays for each line. For the ancestor, the mean is based on two replicate assays for each of the two marker variants. A malthusian parameter of 0 day^{-1} indicates persistence in daily serial dilution culture; −4.6 day^{-1}indicates that the population is declining due to dilution without any growth or death.

ability levels corrected using the sequential Bonferroni technique (Rice 1989). The increase in the final fitness of the 20°C group, representing adaptation to a novel environment, was significantly higher than was observed for the 37°C group, which was maintained in the ancestral environment. The fitness of the 20°C group was, however, significantly *lower* than the 42°C and 32–42°C groups even after applying the sequential Bonferroni correction to the probability level. There was no significant difference between the fitness response at 20°C and that at the more moderate (but also novel) temperature of 32°C. With respect to the rates of adaptation, only the comparison with the 42°C group was significant. However, this comparison is the one of primary interest with respect to the hypothesized effect of the niche edge. Neither the rate nor final extent of adaptation supports the hypothesis of a response to selection at the lower thermal niche boundary comparable in magnitude to that at the upper niche boundary. Rather, there appears to be an asymmetrical response to selection at the upper and lower boundaries, with the high rate of change in fitness that was previously observed (Bennett et al. 1992) being unique to the upper thermal boundary.

Thermal Niche Modification.—The absolute fitnesses of the six lines of the 20°C group and their ancestor are plotted as a function of temperature in Figure 3. The absolute fitness of the ancestor was compared with the estimates for the lines of the 20°C group by a Mann-Whitney test at assay temperatures near the boundaries of the ancestral thermal niche. All six lines are able to maintain their population at a temperature of 18°C, 1.5°C below the ancestral limit. The difference between the 20°C group (based on six replicate lines) and their ancestor (based on the two replicate ancestors of opposite marker types) was significant ($P = 0.046$) at that temperature. At 16.8°C, neither the 20°C group nor the ancestor is able to maintain their population density in this serial dilution regime, but the former has a greater absolute fitness than does the ancestor ($P = 0.046$, with a mean difference of 1.14 day^{-1}). At the upper niche boundary, there is considerably more variation in fitness among the six replicate 20°C-selected lines. At 41.5°C, the absolute fitness of the 20°C group as a whole does not differ from the ancestor ($P = 0.096$); however, four of the six lines are unable to maintain their population density at that temperature. At 42°C, the ancestor is able to sustain sufficient growth to replace its population every 24 h, but all six of the experimental lines are unable to persist; this difference is significant ($P = 0.046$) by the Mann-Whitney test. In the case of at least two of the six lines, the rate of extinction was even greater than the dilution rate during serial transfer, indicating that these lines were not only unable to grow but also must have experienced some cell death at that temperature.

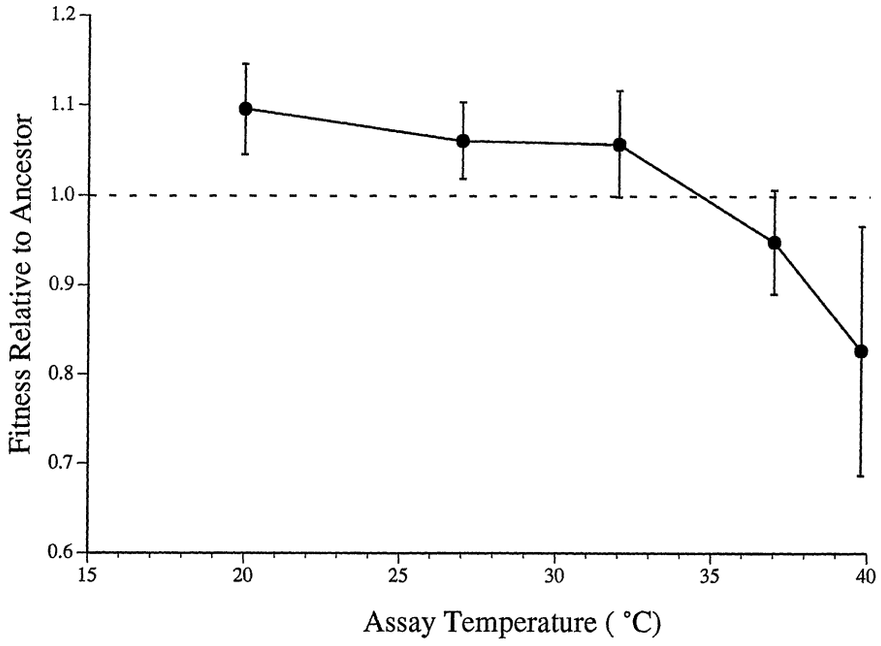

FIG. 4. Mean relative fitness of the 20°C group when assayed at temperatures ranging from 20–39.8°C. Data points represent the means for the six lines and error bars indicate the 95% confidence intervals based on a two-tailed t-distribution with $n - 1 = 5$ degrees of freedom.

Correlated Fitness Responses.—The mean relative fitness of the 20°C group was measured after 2000 generations at temperatures spanning the original thermal niche of the ancestor (Fig. 4). Correlation coefficients between mean relative fitness and assay temperature were calculated for each of the six lines. Three of the six correlations were significant ($P < 0.05$; five temperatures and $n - 2 = 3$ df). This result suggests that most of the adaptation was specific to growth at lower temperatures rather than to the general culture conditions. In fact, adaptation of the 20°C group resulted in a significant decrease in fitness at 40°C (W = 0.83 ± 0.05 SE; two-tailed $P = 0.024$).

Effect of Thermal History.—The fitness responses observed over the course of 2000 generations for the four groups with histories of evolution at different temperatures (32/20, 37/20, 42/20, and 32–42/20°C groups) are shown in Figures 5A–D. A linear model was employed, as described above for the 20°C group, to estimate the rate of change in fitness for each line during this time period. Because these lines were derived from the common ancestor 2000 generations before the start of this experiment, we could not assume that their initial fitnesses at 20°C were equal to the ancestor. Therefore, relative fitnesses at 20°C at time 0 were not constrained to 1.0. The means and standard errors of these rates are summarized in Table 2. The standard errors are calculated based on the six replicate lines within each group. All four groups underwent statistically significant gains in relative fitness at 20°C. An analysis of variance failed to detect any heterogeneity in the rate of change in fitness among the four groups ($F_{3,20} = 0.423$; $P = 0.74$). Inclusion of the 20°C group in this analysis likewise fails to detect significant differences among rates of adaptation ($F_{4,25} = 2.381$; $P = 0.08$).

DISCUSSION

An organism's potential niche is determined by the range of different environmental factors (e.g., temperature, pH, and salinity) in which a population can grow and persist. Suboptimal conditions in environments at the periphery of an organism's normal range or during episodes of environmental change are often physiologically stressful. Parsons (1987) suggested that abiotic, particularly climatic, stresses magnify phenotypic variation and can result in elevated rates of evolutionary change. The ability to maintain replicated populations of bacteria in well-controlled environments for evolutionarily relevant lengths of time has allowed us to make detailed observations of this phenomenon. In addition, we have been able to investigate the nature of genetic correlations between performance in different environments. That is, we can ascertain whether adaptation to a marginal environment will influence an organism's performance within its ancestral range as well as its potential for future adaptation to a changing environment.

Ambient temperature is an important environmental factor even for enterobacteria, which must be capable of transmission to new hosts and therefore persist for extended periods of time outside their host environment. In addition, the enteric bacteria are resident in many host species with a relatively wide range of body temperatures. Bennett et al. (1992) employed natural selection in the laboratory to study adaptation of initially identical populations of *E. coli* to several environments that differed only in temperature. They observed a significantly more rapid response to natural selection at a temperature very near the upper limit of thermal tolerance (42°C) when compared with the response to selection at a

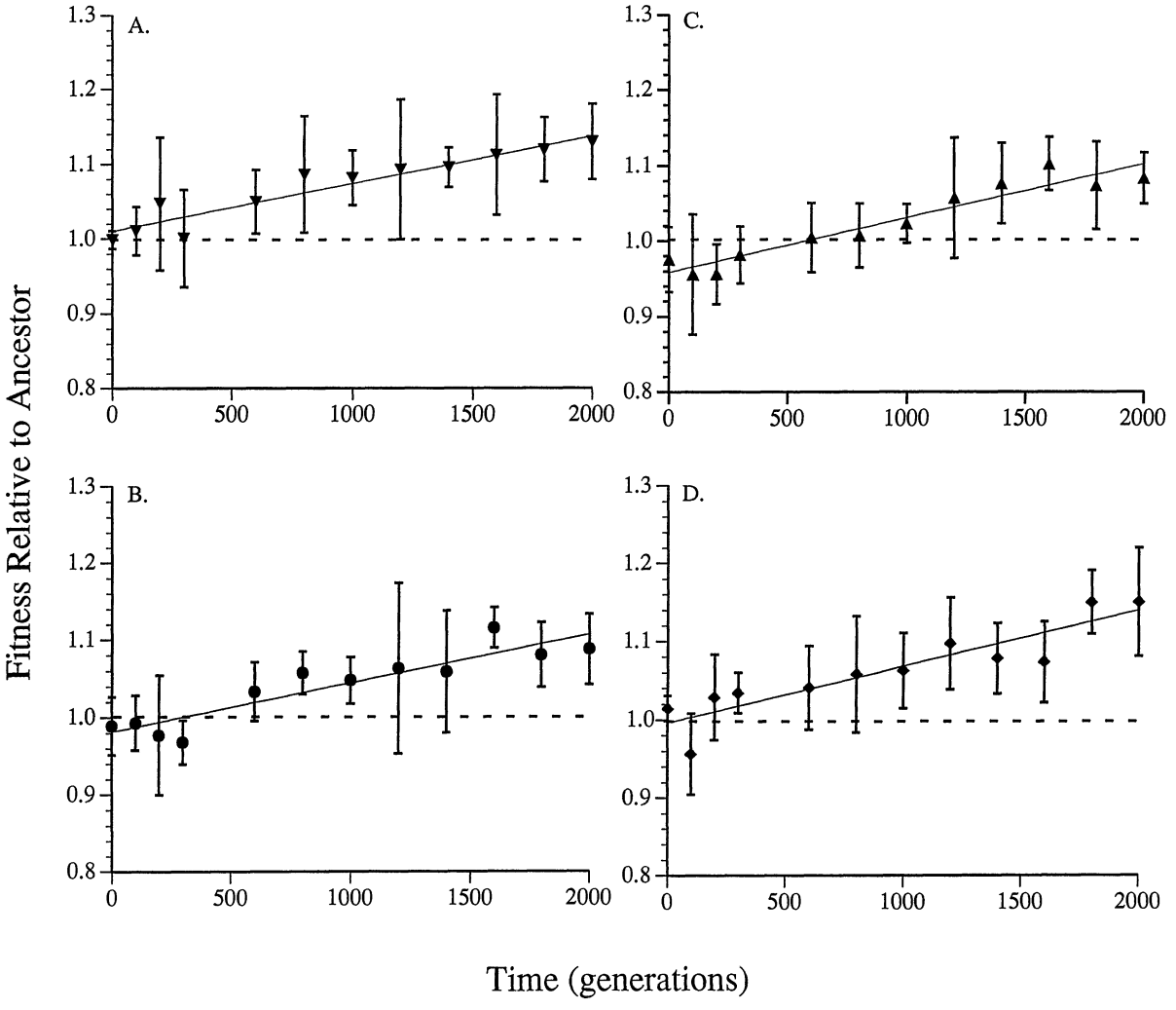

FIG. 5. Fitness response over 2,000 generations of evolution at 20°C for the (A) 32/20, (B) 37/20, (C) 42/20, and (D) 32–42/20°C groups. Fitness of single clones isolated from the evolving populations were estimated relative to the common ancestor at each time point. Each point is the mean fitness of the six replicate lines and the error bars represent the 95% confidence intervals based on a two-tailed t-distribution with $n - 1 = 5$ df. The solid line represents the average linear regression fit to the data points of each of the six lines individually.

moderate temperature (32°C), even though both temperatures were equidistant from the ancestral temperature (37°C). Despite an average increase in fitness of almost 50%, the group of lines evolved at 42°C showed no significant loss of fitness at much lower temperatures (Bennett and Lenski 1993).

TABLE 2. Rates of change in fitness (relative to the common ancestor of all lines) for each group. The rate of change (per generation) for each line was obtained by a linear regression of fitness over time for the entire 2000 generation period.

Experimental group	Mean (± SE)[1]	Significance[2]
32/20°C	$6.40 (\pm 0.72) \times 10^{-5}$	<0.001
37/20°C	$6.32 (\pm 0.64) \times 10^{-5}$	<0.001
42/20°C	$7.23 (\pm 0.62) \times 10^{-5}$	<0.001
32-42/20°C	$7.17 (\pm 0.97) \times 10^{-5}$	<0.001

[1] Mean (and standard error of the mean) based on 6 replicate lines for each experimental group.

[2] One-tailed probability, using the t-distribution with n-1 = 5 df; the null hypothesis is that the mean rate of change equals 0.

The current study, designed to test the generality of the previous results by repeating the experiment near the lower thermal niche boundary (20°C), produced quantitatively and qualitatively very different results. These may be summarized in three major conclusions. First, occupation of a habitat in which the environment is marginal for persistence of a population is not a sufficient condition to explain an elevated rate of adaptation. During 2000 generations of evolution at 20°C, the bacterium in this study adapted to its environment with an average increase in fitness relative to the common ancestor of only 8%. The average rate of increase in fitness at this temperature was not significantly different from that observed at a novel temperature much closer to the middle of the ancestral thermal niche (32°C) and was significantly lower than that observed near the upper thermal limit (42°C). Our results thus indicate that neither simple distance from the ancestral environment nor proximity to the edge of the niche are sufficient indicators of exceptionally high rates of evolutionary change.

The second conclusion is that performances at higher and lower temperatures in the 20°C lines are genetically correlated. Associated with the increase in relative fitness at 20°C was a decrease in fitness at higher temperatures (by 17% when assayed at 40°C). In addition to the relative fitness response, both the upper and lower limits of thermal tolerance, as measured by the ability of a population to persist in the serial transfer environment, shifted downward by 1–2°C. These observations of trade-offs associated with adaptation to 20°C are again in contrast to the lack of any general observation of trade-offs associated with adaptation to higher temperatures (Bennett et al. 1992; Bennett and Lenski 1993). A related study in *Drosophila melanogaster* (Huey et al. 1991) demonstrated a correlated effect of natural selection at intermediate temperatures on tolerance to heat shock, however, they observed a significant *increase* in tolerance to high temperatures rather than a trade-off. Many evolutionary ecology models (e.g., Lynch and Gabriel 1987) assume that trade-offs will be associated with adaptation. Our results illustrate that any assumptions about trade-offs may depend not only on the particular environmental factor that is acting as the selective agent but even on the direction of change in that environmental factor.

We have defined the thermal niche of a population as the range of temperatures over which it can sustain itself indefinitely, given a particular set of other environmental conditions including resource level and density-independent mortality (due to serial dilution). Evidently, the evolutionary responses to the lower and upper edges of the thermal niche are quite different, both in terms of its rapidity and the effects of that evolution on the thermal niche itself. Why were there these differences? We can only speculate, but it may be important that the upper boundary is extremely sharp and characterized by a sudden shift from rapid growth to marked death between 42 and 44°C (see Fig. 4 in Bennett and Lenski 1993). By contrast, the lower niche boundary is characterized by a gradual reduction in growth rate, which at some point simply becomes insufficient to offset the losses due to serial dilution. That is, in contrast to temperatures just beyond the upper edge, there does not appear to be any sudden dramatic change in cell physiology just below the lower edge.

A third conclusion is that, despite our demonstration of genetic correlations between performance at different temperatures, there was no discernible effect of selection history on adaptation to this novel thermal environment. Other studies have shown that responses to uniform artificial selection can amplify historical differences in the value of some traits between samples from natural populations. For example, populations of *D. melanogaster* and *Drosophila pseudoobscura* derived from different localities exhibited heterogeneous responses to artificial selection for ethanol knockdown resistance and correlated traits (Cohan and Hoffmann 1986; Hoffmann and Cohan 1987). While 2000 generations may not have been long enough for strong historical contingencies to develop in our bacterial lines, we have demonstrated elsewhere that a trait that is not significantly correlated with Darwinian fitness was more influenced by historical contingencies than was fitness itself (Travisano et al. 1995).

Asymmetries in Evolutionary Responses.—The different responses to natural selection at the upper and lower thermal niche boundaries of the *E. coli* populations described here provide a new example of an asymmetrical evolutionary response to selection in alternate environments. Other examples include an experimental evolution project involving adaptation of bacterial populations to alternate resources (Travisano 1993). In that case, adaptation to growth in a glucose-limited environment resulted in significant variation in fitness in a maltose-limited environment. Adaptation to growth in the maltose-limited environment, on the other hand, resulted in no increased variation in fitness in glucose. A third example is derived from data on parasitic wasps, *Aphytes*, artificially selected for heat and cold tolerance (White et al. 1970; Huey and Kingsolver 1989). Selection for cold tolerance increased both cold and heat tolerance, whereas selection for heat tolerance increased only heat tolerance with no effect on cold tolerance. In addition to laboratory studies, a comparison of prokaryotes isolated from high and low temperature environments suggests that such asymmetries occur in response to selection pressures in natural environments as well. Many of the prokaryotes isolated from high temperature environments are obligate thermophiles, whereas those resident in low temperature environments are not obligate psychrophiles and generally grow optimally at much higher temperatures (Knoll and Bauld 1989). Whether these differences reflect past selective pressures during the course of Earth's history or underlying asymmetry in the connections between metabolic processes that are important at high and low temperatures is only speculation at this point. If the latter is true, are these asymmetries the result of inherent physical or biochemical constraints or has past selection favored an asymmetric design of the metabolic architecture? A corollary to this study might be to look at correlated effects of temperature-sensitive mutations on fitness at other temperatures.

Charting the path between the phenotypic adaptations responsible for fitness differences and the underlying biochemical and genetic changes that have occurred is likely to be difficult. Dykhuizen and Dean (1990), however, demonstrated that a simple model mapping enzyme kinetic parameters to fitness for the lactose operon could account for seemingly complex phenomena such as genotype-by-environment interactions. Using their model, one can see how those genotype-by-environment interactions can in turn influence the order in which particular metabolic steps are targeted by natural selection. Thus, the asymmetrical nature of our observations on thermal adaptation may hint at further information regarding the underlying metabolic architecture that is the source of the phenotypic differences.

Finally, an analysis of the phenotype of experimentally evolved lines in different environments has the potential to lead to identification of the molecular basis of the adaptations. Further analyses of these lines promises to expand our understanding of natural selection to include not just the results of selection but also the nature of the genetic variation and its relationship to the phenotypic variation upon which selection operates.

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