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EVOLUTIONARY ADAPTATION TO TEMPERATURE II. THERMAL NICHES OF EXPERIMENTAL LINES OF *ESCHERICHIA COLI*

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Abstract.—Groups of replicated lines of the bacterium *Escherichia coli* were propagated for 2,000 generations at constant 32, 37, or 42°C, or in an environment that alternated between 32 and 42°C. Here, we examine the performance of each group across a temperature range of 12–44°C measuring the temperatures over which each line can maintain itself in serial dilution culture (the thermal niche). Thermal niche was not affected by selection history: average lower and upper limits remained about 19 and 42°C for all groups. In addition, no significant differences among groups were observed in rate of extinction at more extreme temperatures. Within the thermal niche, we measured the mean fitness of the evolved groups relative to their common ancestor. Increases in mean fitness were temperature specific, with the largest increase for each group occurring near its selected temperature. Thus, the temperature at which mean fitness relative to the ancestor was greatest (the thermal optimum) diverged by about 10°C for the groups selected at constant 32°C versus constant 42°C. Tradeoffs in relative fitness (decrements relative to the ancestor elsewhere within the thermal niche) did not necessarily accompany fitness improvements, although tradeoffs were observed for a few of the lines. We conclude that adaptation in this system was quite temperature specific, but substantial divergence among groups in thermal optima had little effect on the limits of their thermal niches and did not necessarily involve tradeoffs in fitness at other temperatures.

Key words.—Adaptation, bacteria, *Escherichia coli*, fitness, temperature, thermal niche, thermal optimum, tradeoff.

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When a population encounters a novel environment, it may adapt genetically to these new circumstances, provided that there is sufficient time and genetic variation and that the population does not go extinct. Adaptation here refers specifically to an improvement in fitness of the population relative to its original (= ancestral) condition when it first entered the new environment. The consequences of this adaptation for functional capacities and fitness in other environments are less predictable. For example, are there tradeoffs in performance or fitness in other environments during adaptation to one specific environment? Does adaptation produce shifts in the environmental range tolerated, such that the population may thereby become capable of functioning in environments that it could not tolerate ancestrally and which it has not actually experienced? In other words, does the evolving population become preadapted to even more extreme environments, even if it has not encountered those environments?

Consider adaptation to a new thermal en-

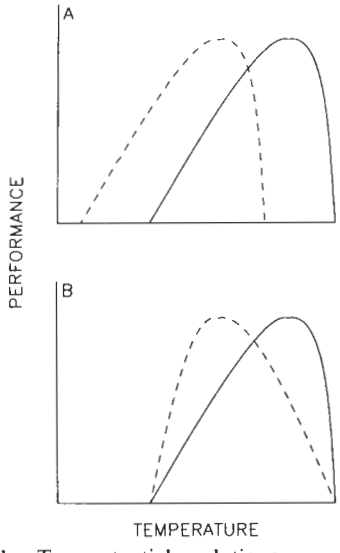

FIG. 1. Two potential evolutionary responses following a shift from a warmer (solid line) to a cooler (dashed line) environment. In panel A, the entire performance function, including limits and optimum (sensu Huey and Stevenson, 1979), is translated to lower temperatures. In panel B, the optimum is shifted but the limits are not.

vironment by a population that has experienced a moderate temperature for a long period and has become genetically adapted to that temperature. Then temperature decreases and the population begins to adapt to the cooler temperature. Does this adaptation improve fitness in all environments cooler than the ancestral? Does it decrease fitness in the ancestral environment and in all environments warmer than the ancestral? Does it extend the range of cold environments that can be tolerated by the population? Does it decrease the highest temperature that the population can tolerate, that is, does the population lose heat-tolerance? Two potential evolutionary outcomes are shown in Figure 1. In the first case (Fig. 1A), the range of temperatures tolerated shifts downward. In the second (Fig. 1B), the range does not shift, but performance at the new environmental temperature improves while performance decreases at the ancestral temperature. These are only two of many possible outcomes (Huey and Kingsolver, 1989), and a similar series can be envisioned for adaptation to warmer temperatures.

Comparative information on species living in different thermal environments is available for many types of physiological processes, such as metabolic rate, heart rate, and running speed (e.g., Hochachka and Somero, 1984; Prosser, 1986; Cossins and Bowler, 1987). The pattern typically observed more closely approximates Figure 1A than Figure 1B. That is, critical thermal limits generally shift during thermal adaptation, even if thermal niche breadth is not strictly conserved (e.g., Brett, 1970; Huey, 1982; Huey and Bennett, 1987; Huey et al., 1991). Interpretation of such data, however, is complicated by several factors, including the diverse phylogeny of the animals examined, other environmental components in addition to temperature, and the unknown fitness consequences of the variables measured. To address these issues, we have undertaken an experimental laboratory study of evolutionary adaptation of *Escherichia coli* to different thermal environments (Bennett et al., 1990, 1992). This study reports the effects of that thermal adaptation on the range of temperatures tolerated by experimental populations of these bacteria and on relative and absolute fitnesses at temperatures within and beyond this range.

EXPERIMENTAL OVERVIEW

We have previously described the design and some results of an earlier experiment in which bacteria evolved in the laboratory under several different thermal regimes (Bennett et al., 1992). A common ancestral genotype of *E. coli* was used to found six replicate *lines* for each of four treatment *groups*: constant 32, 37, and 42°C, and alternation between 32 and 42°C. The common ancestor had previously been selected at 37°C for 2,000 generations (Lenski et al., 1991). These 24 lines were propagated for an additional 2,000 generations at their respective thermal regimes, but all other aspects of the culture conditions were identical. The common ancestor was maintained in a nongrowing state at −80°C. Evolutionary adaptation was measured as changes in mean fitness relative to the common ancestor, as determined by competition experiments at various temperatures. Two types of evidence indicate that the adaptation of these groups was, in fact, largely tem-

perature specific (Bennett et al., 1992). First, the increase in relative fitness at the selected temperature of each group was greater in the novel thermal regimes (32, 42, and 32/42°C) than at the ancestral temperature (37°C). Second, these direct responses of relative fitness were usually greater than were correlated responses at other temperatures.

Here, we examine more broadly the correlated responses of the selected groups, including aspects of performance outside as well as within each line's thermal niche. Here we define the *thermal niche* of a genotype as the range of temperatures over which that genotype can replace itself, when other environmental parameters are held constant. Thermal niche is measured in this experimental system as the temperatures over which the Malthusian parameter is zero (i.e., the population density remains constant over time). Outside its thermal niche, a genotype's *absolute fitness* is insufficient to sustain itself, even in the absence of competing genotypes, and its population density declines towards extinction at a rate that is governed by its Malthusian parameter at that temperature. At temperatures that are within the thermal niche of both a derived genotype and its ancestor, changes in performance are expressed as *relative fitness,* measured by direct competition experiments. We define operationally the *thermal optimum* of each group as that temperature at which its mean fitness relative to the common ancestor is greatest.

Specifically, we seek to answer the following set of questions: 1) What is the thermal niche of the common ancestor? That is, what are the upper and lower thermal limits that permit maintenance of an ancestral population, in the absence of competition with other genotypes? 2) Have these limits changed in the groups that evolved for 2,000 generations under the various thermal regimes? If so, was the breadth of the thermal niche malleable or conserved? 3) How concordant in magnitude are changes in thermal optima and in thermal limits for the selected groups? For example, are 5°C shifts in thermal optima accompanied by comparable shifts in the upper and lower limits of the thermal niche? 4) Were improvements in fitness in one portion of the thermal niche accompanied by decrements in other portions? That is, were tradeoffs in fitness involved during thermal adaptation?

Materials and Methods

Bacteria.—All of the genotypes used in this study have been described previously (Bennett et al., 1992). Briefly, the common ancestor has two forms, Ara− and Ara+, which can be distinguished from one another by their colony color on tetrazolium arabinose (TA) indicator agar. Twenty-four independently derived lines were isolated as clones after 2,000 generations (300 days) of propagation at their respective thermal regimes. All the ancestral and derived genotypes are stored indefinitely at −80°C, so that their properties can be directly compared at any time. We usually refer to the mean properties of groups but occasionally identify specific genotypes by culture number in a frozen collection.

Culture Conditions.—The basic culture conditions used in this study have been described previously (Bennett et al., 1992). Briefly, bacteria are removed from the freezer, cultured for one day in Luria broth (LB), and propagated by daily serial transfer (100-fold dilution) in glucose-limited (25 microgram ml^{-1}) Davis minimal medium (DM) for the duration of an experiment. The same culture conditions are used in assaying relative and absolute fitnesses (see below) as were used during the 2,000 generations of experimental evolution, except culture temperature as indicated. Experimental temperatures were maintained within ~1°C of the target in all experiments by using the thermostatic controls built into the shaking air-bath incubators (New Brunswick models G25 and G25-KC), coupled with the placement of culture flasks in central positions to avoid internal gradients that existed in the incubators. We monitored temperatures using a Tegam model 821 electronic thermometer and thermocouples calibrated to within 0.2°C using a thermometer referable to standards of the National Institute of Standards and Technology. The thermocouples were immersed in water-filled flasks identical to and adjacent to those used to propagate the bacteria. In certain experiments conducted at high temperatures (41–43°C), we obtained very fine thermal control by taking advantage of

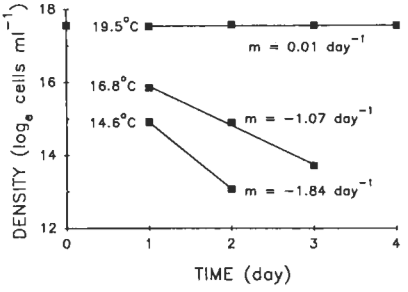

FIG. 2. Population density trajectories of the ancestral genotype (REL1206) in serial dilution culture at three different temperatures. m = Malthusian parameter, estimated as the rate of change in population density with time.

consistent thermal gradients that existed within an incubator.

Measurements of Absolute Fitness.—Absolute fitnesses of each ancestral and derived genotype were measured at the following temperatures: 12.0, 14.6, 16.8, 19.5, 22.3, 39.8, 42.0, and 43.8°C. We also estimated the absolute fitnesses for four genotypes at a much finer resolution between 41 and 43°C. Each line was inoculated from its freezer vial into LB and incubated at 37°C for 24 hr. This culture was then diluted 1:10^4 into DM and incubated at 37°C for another 24 hr. The density of this "day zero" culture was determined as described below; the culture was diluted 1:100 into fresh DM and incubated at the experimental temperature for 24 hr. The density of this "day one" culture was determined, and the culture was similarly diluted and incubated for as many as four days at the experimental temperature.

Absolute fitness was estimated by regressing the natural logarithm of density against time. The slope of the regression is equivalent to a genotype's Malthusian parameter. If the genotype can sustain a constant population density at the experimental temperature (given the composition of the medium and the imposed dilution factor), then the slope of this line equals zero, within statistical accuracy. Note that a constant population size requires ~6.64 (= $\log_2$ 100) generations of binary fission during each 24 hr period to offset the 100-fold dilution. The Malthusian parameter cannot be significantly greater than zero, owing to the density-dependent resource limitation inherent in this system. A Malthusian parameter significantly less than zero implies that population density is declining towards extinction. A Malthusian parameter of $\log_e$ 0.01 = −4.6 day^{-1} would be obtained for a population that neither reproduced nor died, but was diluted from culture. Figure 2 illustrates the calculation of the Malthusian parameters for one genotype at three different experimental temperatures. The number of points included in each regression is limited by the number of time points yielding satisfactory estimates of population density (see below). Whenever two or more later points were available, the "day zero" point was excluded from the regression, because (i) the stationary-phase density (cell yield) may differ between 37°C and the experimental temperature and (ii) a transient effect may be associated with the shift from 37°C to the experimental temperature.

Population densities were estimated electronically and by plate counts. Electronic counts were obtained using a Coulter Counter model ZM that was equipped with a 30-micron diameter aperture and connected to a Coulter Channelyzer model 256. DM cultures were diluted 100-fold into Fisher isotonic dilutent, and 0.1 ml of the resulting dilution was electronically counted. Particles having an estimated volume less than 0.25 femtoliter (= 0.78 micron effective diameter for an equivalent sphere) were excluded. This criterion generally coincided with a clear separation between cells and very small background particles that were always present. We also subtracted counts that were obtained from 100-fold dilutions of sterile DM cultures into the isotonic dilutent to eliminate any larger background particles. Plate counts were obtained by standard dilutions and spreading on TA agar, with plates incubated at 37°C regardless of experimental temperature. Below 40°C, population densities estimated electronically and by plate counts agreed reasonably well with one another. To avoid biases that might arise from using different counting methods, the Malthusian parameters at any given temperature were calculated using only one type of data for all genotypes, the "day zero" points were always excluded from the regressions, and the same number of time points were used in the regressions for all genotypes. At higher tem-

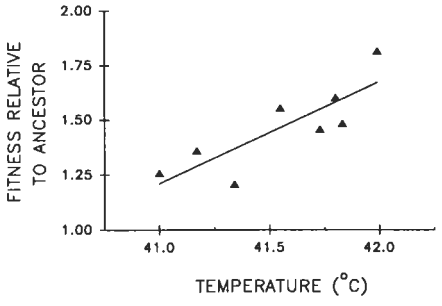

FIG. 3. Relative fitness of one line (REL2047) of the 42°C evolved group in competition with the common ancestor along a 1°C thermal gradient. Symbols are independent fitness estimates from flasks along the gradient; line is least-squares regression ($r = 0.83$, $n - 2 = 5$ *df*, $P < 0.05$).

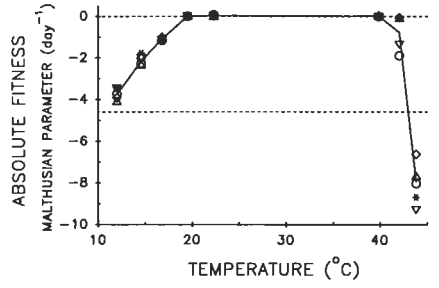

FIG. 4. Mean absolute fitnesses of the common ancestor (asterisks), 32°C group (downward triangles), 37°C group (circles), 42°C group (upward triangles), and 32/42°C group (diamonds) between 12 and 44°C. Malthusian parameter = 0 day^{-1} indicates persistence in daily serial dilution culture; = −4.6 day^{-1} corresponds to dilution without any growth or death. Means are calculated from the six replicate lines within each group (except the common ancestor, for which the mean is based on the two marker variants). Absolute fitness between groups did not differ significantly at any assay temperature (see text).

peratures, however, these criteria could not be applied for several reasons. First, population density sometimes dropped so precipitously that the "day zero" points could not be excluded. Second, plate and electronic counts sometimes showed discrepancies, with the plate counts systematically lower. This presumably resulted from the counting of inviable cells by the electronic method. In this case, densities were based on plate counts. Third, population densities sometimes recovered following precipitous declines (dubbed the "Lazarus" effect). These recoveries apparently resulted from the proliferation of mutants that had an extended upper thermal limit for population persistence. Consequently, we excluded these increments from our calculations.

Measurements of Relative Fitness.—Estimates of relative fitness were obtained within the common thermal niche of the ancestral and derived lines using the methods of Bennett et al. (1992). Briefly, each derived genotype was allowed to compete against the reciprocally marked ancestral genotype at an experimental temperature. Immediately prior to competition, the two competitors were grown separately for one day in DM at the experimental temperature, so that both were acclimated to the culture conditions. They were then each diluted 1:200 into the same flask containing fresh DM, a sample was immediately taken to determine their initial densities by plating on TA agar, and the competition culture was incubated at the experimental temperature for 24 hr. At the end of this period, another sample was taken to determine the final densities of the competitors, again by plating on TA agar. Relative fitness is calculated as the ratio of the number of doublings achieved by the derived and ancestral genotypes over one day. Relative fitness measurements at 32 and 37°C are taken from Bennett et al. (1992). Because of the strong thermal dependence of fitness around 42°C (Fig. 3) and in view of the greater temperature control achieved in this study (see above), new experiments were performed at 39.8, 41.0, and 41.8°C, as well as at 19.5, 22.3, and 27.0°C.

RESULTS

Absolute Fitness and the Thermal Niche.—Figure 4 showed the mean absolute fitness (Malthusian parameter) for each evolved group and for the common ancestor at temperatures from 12 to 44°C. Kruskal-Wallis tests (Sokal and Rohlf, 1981) were performed to assess heterogeneity among the four groups (32, 37, 42, and 32/42°C) in absolute fitness at each assay temperature. None of these seven tests was statistically significant ($P > 0.1$ in all cases), indicating that the four groups had not diverged appreciably in absolute fitness at any temperature.

The thermal niche of a genotype is defined here as the range of temperatures over which it can maintain constant population

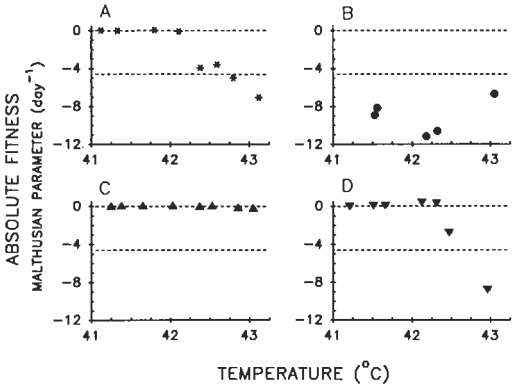

FIG. 5. Absolute fitnesses of four genotypes between 41 and 43°C. (A) Common ancestor (REL1206); (B) one of six lines evolved at 37°C (REL2044); (C) one of six lines evolved at 42°C (REL2047); (D) one of six lines evolved at 32°C (REL2037). See Figure 4 for an explanation of values shown by dashed lines.

density by replicating sufficiently to offset dilution plus any cell death. Because the Malthusian parameter is estimated empirically and therefore has an associated sampling error, we chose a minimum value of -0.2 day^{-1} to satisfy the maintenance condition. In choosing this value, we took advantage of the fact that an ideal population can have a Malthusian parameter less than or equal to zero, but not greater than zero owing to the density dependence in our experimental system. Experimental populations rarely produced estimates of the Malthusian parameter that were, by chance, greater than $+0.2$ day^{-1}, and so we assumed that an estimate less than -0.2 day^{-1} indicated a real failure of the population to persist rather than mere sampling error. In any case, few populations yielded estimates of the Malthusian parameter that were near this cut-off, and most had estimates well above or below it.

The ancestral genotype persisted at 19.5 and 42°C, but it failed to persist at either 16.8 or 43.8°C (Fig. 4). A finer mapping of persistence of the ancestral genotype indicates an upper thermal limit of about 42.2°C (Fig. 5A). The ancestral thermal niche is therefore 19 to 42°C. Every line in each evolved group also maintained itself between 19 and 40°C, whereas none persisted below 19°C. At 43.8°C, none of the lines in any of the groups persisted. At 42°C, all of the lines in the 42 and 32/42°C groups persisted, but only some of the 32 and 37°C lines were able to do so. These results suggest that some lines may have upper thermal limits for persistence that are slightly different from that of the common ancestor, even though the groups did not differ significantly in absolute fitness at any temperature.

The absolute fitnesses of four particular genotypes were estimated along a slight but well-defined thermal gradient that existed in the incubator. The ancestral genotype persisted at 42.1°C, but it did not persist at 42.3°C (Fig. 5A). By contrast, one of the 37°C lines failed to persist even at 41.5°C (Fig. 5B), whereas one of the 42°C lines persisted to at least 43°C (Fig. 5C). None of the other five 42°C lines, however, was able to persist at 43°C (data not shown). Finally, one of the 32°C lines responded very similarly to the ancestral genotype, indicating that even a slight contraction of the upper thermal limit was not a necessary consequence of prolonged adaptation to a lower temperature (Fig. 5D). Evidently, some lines showed slight (1–2°C) contractions or extensions of their upper thermal limit in comparison to their common ancestor, although the groups with different selective histories could not be statistically distinguished on this basis. Those few lines that exhibited slight changes in their upper thermal limits gave no indication of opposing changes in their lower thermal limits, so that niche breadth also must have differed slightly between lines (but not groups). Even these very small changes in the thermal niche, however, were not a necessary correlate of adaptation to the different thermal regimes.

Between 12 and 19°C, growth of the populations—although evident—was insufficient to produce the ~6.6 doublings required to offset the 100-fold daily dilution. Growth ranged from an average of 1.2 doublings day^{-1} at 12°C to 5.1 doublings day^{-1} at 16.8°C, and had a temperature coefficient, Q_{10}, of 20.4. Extinction at these lower temperatures is thus a population phenomenon resulting from the imposed culture regime, not a complete failure of the bacteria to endure these temperatures. By contrast, absolute fitness drops precipitously just beyond the upper boundary of the thermal niche (Figs. 4 and 5). For example, at 43.8°C—less than 2°C beyond the upper

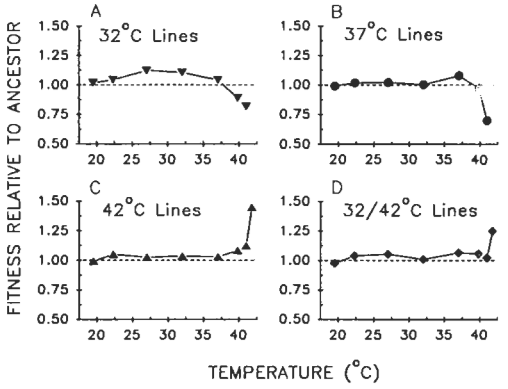

FIG. 6. Mean fitnesses of the evolved groups relative to the common ancestor, as assayed by competition experiments within their mutual thermal niche. (A) 32°C group; (B) 37°C group; (C) 42°C group; (D) 32/42°C group. Means are calculated from the six replicate lines within each group. Statistical analyses are presented in Table 1.

thermal limit for most lines—cell populations disappeared from culture more rapidly (average Malthusian parameter for all genotypes = -8.0 day^{-1}) than could be accounted for by serial dilution alone ($\log_e 0.01 = -4.6$ day^{-1}). Thus, high temperatures not only inhibited growth but were lethal.

Relative Fitness and the Thermal Optimum.—Figure 6 shows the mean fitness of each evolved group relative to the common ancestor across the range of temperatures that encompasses their mutual thermal niche. The thermal specificity of adaptation is quite striking and is supported by statistical analyses (Table 1). The group that evolved at 32°C improved significantly at 27 and 32°C, but showed no significant improvement at 22°C, 37°C, nor at any more extreme temperature (Fig. 6A). The 37°C group was significantly more fit than the common ancestor at 37°C, but not at 32°C, 40°C, nor at more extreme temperatures (Fig. 6B). The 42°C group improved significantly at 40, 41 and 42°C, but not at 37°C nor at any lower temperature (Fig. 6C). The thermal dependence of this group's fitness function was especially pronounced, increasing from a mean of 1.12 at 41°C to a mean of 1.45 at 41.8°C ($Q_{10} = 24.0$). Finally, the group that evolved in the thermal regime that alternated daily between 32 and 42°C improved significantly in that alternating regime (Bennett et al., 1992), as well as over a broad range of temperatures: 22, 27, 32 (A. M. Leroi, R. E. Lenski, and A. F. Bennett, in prep.), 37 and 42°C (Fig. 6D).

We have defined the thermal optimum of a group to be that temperature at which mean fitness relative to the common ancestor is greatest. The thermal optima for the groups evolved at constant 32, 37, and 42°C are approximately 30, 37, and 42°C, respectively (Table 2). Thus, these optima closely match the temperatures at which the groups evolved for 2,000 generations. The thermal optimum for the group that evolved in the alternating regime is, by this definition, also 42°C. However, this group has the broadest range of temperatures over which significant improvements in fitness oc-

TABLE 1. Statistical analyses of the relative fitnesses of the four evolved groups at different assay temperatures. Mean relative fitnesses for each group are plotted in Figure 6.

Assay temperature	Group[1,2]				Heterogeneity among groups[1,3]
	32°C	37°C	42°C	32/42°C	
19.5°C	NS	NS	NS	NS	NS
22.3°C	NS	NS	NS	*	NS
27°C	***	NS	NS	***	**
32°C	*	NS	NS	—[4]	*
37°C	NS	**	NS	**	NS
39.8°C	NS	NS	*	NS	*
41°C	NS	NS	*	NS	**
41.8°C	—[5]	—[5]	*	*	—[5]

[1] Significance is denoted: NS for $P > 0.05$; * for $0.01 < P < 0.05$; ** for $0.001 < P < 0.01$; or *** for $P < 0.001$.
[2] The null hypothesis is that mean fitness relative to the common ancestor equals 1; probabilities calculated using a two-tailed t-test with $n - 1 = 5$ df.
[3] The null hypothesis is that groups do not differ from one another in their fitnesses relative to the common ancestor; probabilities calculated using the Kruskal-Wallis test (Sokal and Rohlf, 1981).
[4] Additional assays performed by A. M. Leroi, R. E. Lenski, and A. F. Bennett (unpubl. data) indicate that the slight fitness improvement of the 32/42°C group at 32°C is significant.
[5] Tests not performed because some lines in the 32 and 37°C groups were outside their thermal niches.

TABLE 2. Summary of approximate thermal limits and optima for experimentally selected groups and their common ancestor.

Group	T_L[1]	T_U[2]	T_O[3]
Common ancestor	19°C	42°C	NA
32°C	19°C	41°C	30°C
37°C	19°C	41°C	37°C
42°C	19°C	42°C	42°C
32/42°C	19°C	42°C	42°C

[1] Lower thermal limit for population persistence. Below the indicated temperature, the mean Malthusian parameter for the lines within a group falls below zero (see Fig. 4).
[2] Upper thermal limit for population persistence. Above the indicated temperature, the mean Malthusian parameter for the lines within a group falls below zero (see Fig. 4).
[3] Thermal optimum. At the indicated temperature, the mean fitness (relative to the common ancestor) for the lines within a group is greatest (see Fig. 6). NA = not applicable.

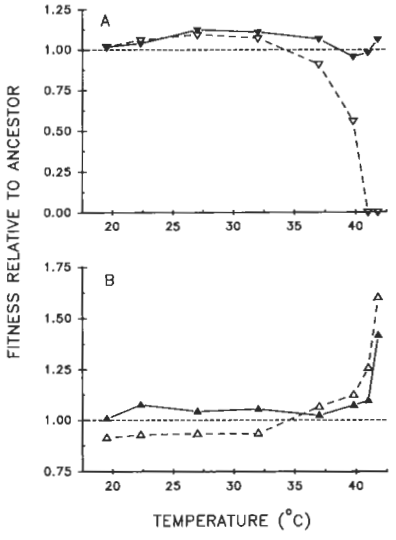

FIG. 7. Heterogeneity among lines within evolved groups, as reflected in fitnesses relative to the common ancestor. (A) Hollow symbols and dashed line correspond to one of six lines in the 32°C group (REL2038); filled symbols and solid line correspond to the mean of the other five lines in the 32°C group. At each of the four highest temperatures, REL2038 had the lowest relative fitness in its group. The probability that any one of the six lines would have the lowest fitness values is <0.005 [$6 \times (1/6)^4$]. (B) Hollow symbols and dashed line correspond to one of six lines in the 42°C group (REL2047); filled symbols and solid line correspond to the mean of the other five lines in the 42°C group. At each of the four lowest temperatures, REL2047 had the lowest relative fitness in its group ($P < 0.005$, see part A).

curred (Table 1). The approximately 10°C divergence of the thermal optima of the evolved groups (Fig. 6) contrasts markedly with the extreme constancy of their thermal niches (Fig. 4).

Whereas each of the groups had significantly improved fitness relative to the common ancestor at certain temperatures, none of the groups exhibited statistically significant tradeoffs in fitness (i.e., decrements relative to the ancestor) at any other temperatures. Even the seemingly large drops in mean relative fitness of the 32 and 37°C groups above approximately 40°C (Fig. 6A and 6B) were not significant, being due to the effects of only a single line of the 32°C group (Fig. 7A) and perhaps three lines of the 37°C group. The absence of fitness tradeoffs in the group evolved in the alternating 32/42°C environment is not surprising, given the selection for thermal generalism. For the 42°C group, however, the lack of any fitness tradeoff at low temperatures is surprising. Figure 7B shows that one of the 42°C lines was less fit than the ancestor at all temperatures below 37°C, and that this same line consistently gave among the highest fitnesses above this temperature. But the other five lines in the 42°C group were, if anything, slightly more fit than the common ancestor even at low temperatures. (Note that the 42°C line exhibiting this tradeoff also was the only line having an extended upper thermal limit, Figure 5C.) Evidently, correlated losses of fitness at dissimilar temperatures may sometimes occur during selection for thermal specialists, but such tradeoffs are not necessary outcomes of temperature adaptation in this system.

"Lazarus" Effect.—During exposure to temperatures above the upper thermal limit for persistence, we occasionally observed the sudden recovery and subsequent maintenance of populations whose densities had initially declined markedly (Fig. 8A). Bacteria isolated at the end of these experiments still possessed genotypic markers characteristic of our lines (see Lenski et al., 1991; Bennett et al., 1992), excluding contamination by more thermophilic bacteria. One plausible explanation for this "Lazarus" effect is that high temperature selected for rare thermophilic mutants, which prospered while other members of the population went extinct. An alternative explanation is that phenotypic acclimation to high temperature was manifest only after a long delay. In the former case, bacteria isolated from a recovered population should maintain their thermophily even after they have been stored in a freezer and raised at moderate temperatures. In the latter case, bacteria thus treated should revert to a thermally sensitive state, so that when challenged again by the same high temperature they should repeat both the decline and recovery of population density that were observed in the original experiment. When such an experiment was performed using a bacterium isolated from the experiment shown in Figure 8A, no transient decline in population density was observed (Fig. 8B: hollow squares). Simultaneous reculturing of the source population from which this "Lazarus" line was derived, however, showed the same magnitude de-

cline observed previously and, moreover, failed to show any recovery during this repetition (Fig. 8B: filled triangles). The phenotypic persistence of thermophily following storage and growth at lower temperatures, coupled with the stochastic appearance of these thermophiles, indicates that the "Lazarus" effect is due to selection for thermophilic mutants. Such selection is apparently capable of rapidly enriching thermophilic mutants that were not detected by prolonged selection at the extreme upper end of the thermal niche. These pronounced reversals of population trajectories were never observed during exposure to temperatures below the lower thermal limit for persistence.

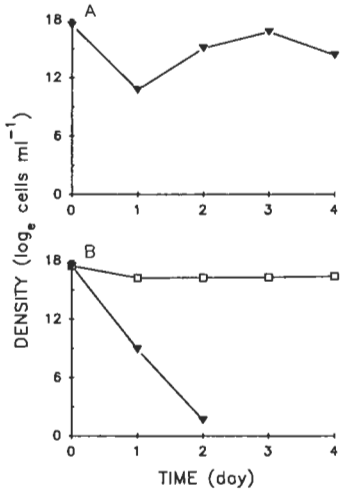

FIG. 8. Recovery of population density at high temperature resulting from hard selection for a thermophilic mutant. (A) One of the lines from the 32°C group (REL2040) declined dramatically during its first day in culture at 43.8°C, but the population then recovered in subsequent days. (B) Open symbols: A bacterial genotype (REL4292), isolated after the recovery shown in panel A, was stored at −80°C, raised at 37°C, and then cultured at 44.0°C, where it exhibited no comparable decline. Closed symbols: The source genotype (REL2040) was simultaneously treated in the same manner, but it exhibited a rapid decline (similar in magnitude to its earlier decline shown in panel A, but without any recovery). See text for further details.

DISCUSSION

Adaptation and Correlated Response.— During 2,000 generations, each group showed considerable adaptation, measured as improvement in competitive fitness, to their novel thermal regimes. For example, fitness of the 42°C group relative to the common ancestor improved 40 to 50% at 42°C. Optimal temperatures for the different experimental groups diverged by as much as 10°C (Fig. 6, Table 2). However, this adaptation was remarkably temperature specific. Neither breadth nor limits of the thermal niche, defined as temperatures at which a population can persist, were significantly differentiated after 2,000 generations of evolution at different temperatures (Table 2). Absolute fitness (Malthusian parameter) outside the thermal niche also was not affected by this adaptation. Tradeoffs in either absolute or relative fitness occurred in only a few of the 24 experimental lines and in none of the experimental groups considered as a whole. The pattern of thermal adaptation in this system was similar to that presented in Figure 1B, except that no decline in performance in the ancestral environment was apparent. Extensive adaptation in this system can clearly occur without consequently compromising performance in other environments. Further, such adaptation does not necessarily open new environments by extending the limits of a niche, even when adaptation occurs very near one of those limits (42°C, in this case). Neither preadaptation to new environments nor loss of function in dissimilar environments is a necessary consequence of such evolution.

This study illustrates the degree to which responses to a continuously distributed environmental variable may be genetically correlated. The fitness function over a range of environmental temperatures might be very rigid, with strong correlations among responses along the entire gradient (Huey and Kingsolver, 1989). If such rigidity is the case, then adaptation to a single temperature will modify performance across the entire gradient. A hypothetical example in which responses in different environments are tightly correlated is shown in Figure 1A: performances at all temperatures change during adaptation to any one. Alternatively, the fitness function might be quite malleable, so that an improvement in performance in one environment has no necessary influence on performance in other environments. Figure 1B presents one such possibility, but the evolutionary malleability of performance could be even more extreme,

with sharp, environmentally specific peaks resulting from adaptation.

In this study, the thermal fitness function (but not the thermal limits) was remarkably malleable. That is, improvements in relative fitness within the niche were highly temperature specific. One example is the 37°C group, which increased fitness at 37°C but did not alter fitness at 40°C, only 3°C away. Likewise, the 42°C group increased fitness at 42°C by 40 to 50% but increased fitness by less than 10% at 40°C. These results suggest that functional traits along a continuously distributed environmental variable are evolutionarily independent and malleable. In its most extreme form, such a function might be regarded as an atomized series of traits, each capable of responding to selection independently. In addition, the area under the fitness function is not constant in these experiments, contrary to the assumption of many ecological and evolutionary models (e.g., Levins, 1968; Huey and Slatkin, 1976; Lynch and Gabriel, 1987; Pease et al., 1989).

Specialists, Generalists, and Thermal Niche Breadth.—Several authors have presented graphical and quantitative models predicting that evolution in temporally varying environments will produce more broadly adapted genotypes than will evolution in constant environments (Levins, 1968; Huey and Slatkin, 1976; Huey and Hertz, 1984; Lynch and Gabriel, 1987). Our bacterial system permits testing of the assumptions and predictions of such models. In this study, we observed no significant changes in the thermal limits for population persistence in the groups evolved at constant 32, 37, or 42°C, nor for the group that evolved in the regime that alternated daily between 32 and 42°C (Fig. 4, Table 2). Consequently, there is no evidence to support a change in thermal niche breadth—defined as the difference between lower and upper thermal limits for population persistence—as the result of selection during 2,000 generations in either constant or varying environments. If, however, one considers the range of temperatures over which fitness relative to the common ancestor has increased, then the group that evolved in the temporally varying thermal regime (32/42°C) appears to have the broadest performance profile. In particular, this group showed statistically significant improvements in fitness at temperatures ranging from 22 to 42°C, whereas no other group showed significant fitness improvement over nearly so wide a range of temperatures (Table 1). However, statistical significance and biological importance must be interpreted cautiously: some of the significant improvements by the 32/42°C group are of similar magnitude to some of the nonsignificant improvements by other groups (Fig. 6). One reason that so many of the responses by the 32/42°C group are significant is that the replicate lines in this group appear to be less variable than in the other groups. In particular, none of the 32/42°C lines shows appreciable losses in absolute or relative fitness at either thermal extreme, as do one or more lines in each of the other groups (Figs. 5B, 7A, and 7B). Thus, not only has the generalist group been selected for improvements at two disparate temperatures, but the performance of the specialist groups has not been subject to selection at one or both thermal extremes.

To understand evolution in temporally varying environments, one may need to take into account not only the range and frequency of alternative environmental conditions, but also the serial correlation of these states. In a forthcoming paper (A. M. Leroi, R. E. Lenski, and A. F. Bennett, in prep.), we will describe phenotypic acclimation following sudden changes in temperature and the consequences of this acclimation for fitness. We will also address how these acclimatory responses evolved during 2,000 generations in the alternating 32/42°C environment.

Hard Versus Soft Selection.—Soft selection occurs when genotypes differ in their relative fitnesses, but selection among the genotypes does not produce any increase in the rate of population growth (absolute fitness) because of the operation of density-dependent factors (Wallace, 1968; Futuyma, 1986). By contrast, hard selection occurs when genotypes differ in their absolute fitnesses, so that changes in genotypic frequencies cause a concomitant increase in the rate of population growth. Hard selection often follows the imposition of some traumatic agent, such as a pesticide, on a

population (see Hoffmann and Parsons, 1991).

Soft selection operated during the 2,000 generation evolutionary experiment that produced the 32, 37, 42, and 32/42°C groups (Bennett et al., 1992) studied here. All four of these thermal regimes permit the indefinite persistence of the ancestral genotype at a constant density, which is limited by the concentration of glucose in the medium. The evolution of fitter genotypes in those regimes had little or no effect on population density. This soft selection was ineffective at extending the thermal limits for population persistence, even when selection operated very near to such a limit and produced a large improvement in relative fitness near that limit. Thus, only one of six lines in the 42°C group—a group that improved dramatically and specifically in its fitness relative to the ancestor at high temperature (Fig. 6C)—extended its upper limit for population persistence and even then by a mere 1°C (to 43°C) (Figs. 4 and 5C).

By contrast, assays of absolute fitness beyond the thermal limits for population persistence represented hard selection. That is, any (initially) rare genotype that could multiply at a sufficient rate at these extreme temperatures would prevent extinction of the population. These hard selection experiments at high temperature sometimes yielded mutants with an extended upper thermal limit (at least 44°C in one instance), and did so in a matter of days (Fig. 8). And yet no mutants with comparably extended upper thermal limits were observed even after selection for 2,000 generations (300 days) at 42°C. These observations suggest that mutants with more pronounced extensions of their upper thermal limit are less fit at 42°C than are the lines evolved at 42°C, although this hypothesis has not yet been explicitly tested. An affirmation of this hypothesis would further support the results from the soft selection experiments, demonstrating that the relative fitnesses of genotypes can exhibit a high degree of temperature specificity.

We plan to use hard selection schemes to see how far the upper thermal limit of this bacterium can be extended. We can then also investigate whether, in extending this limit, tradeoffs in fitness at lower temperatures occur, perhaps even a concomitant increase in the lower thermal limit for population persistence (e.g., Fig. 1A). At present, however, our results based on soft selection better support an alternative model for the evolution of thermal performance (e.g., Fig. 1B).

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Literature Cited

Bennett, A. F., K. M. Dao, and R. E. Lenski. 1990. Rapid evolution in response to high temperature selection. Nature 346:79–81.

Bennett, A. F., R. E. Lenski, and J. E. Mittler. 1992. Evolutionary adaptation to temperature. I. Fitness responses of *Escherichia coli* to changes in its thermal environment. Evolution 46:16–30.

Brett, J. R. 1970. Temperature. Animals. Fishes, pp. 515–560. *In* O. Kinne (ed.), Marine Ecology, Vol. 1. John Wiley, N.Y., USA.

Cossins, A. R., and K. Bowler. 1987. Temperature Biology of Animals. Chapman and Hall, N.Y., USA.

Futuyma, D. J. 1986. Evolutionary Biology, 2nd ed. Sinauer Associates, Sunderland, MA USA.

Hochachka, P. W., and G. N. Somero. 1984. Biochemical Adaptation. Princeton University Press, NJ USA.

Hoffmann, A. A., and P. A. Parsons. 1991. Evolutionary Genetics and Environmental Stress. Oxford University Press, UK.

Huey, R. B. 1982. Temperature, physiology, and the ecology of reptiles, pp. 25–91. *In* C. Gans and F. H. Pough (eds.), Biology of the Reptilia, Vol. 12. Academic Press, N.Y., USA.

Huey, R. B., and A. F. Bennett. 1987. Phylogenetic studies of coadaptation: Preferred temperatures versus optimal performance temperatures of lizards. Evolution 41:1098–1115.

Huey, R. B., and P. E. Hertz. 1984. Is a jack-of-all-temperatures a master of none? Evolution 38: 441–444.

Huey, R. B., and J. G. Kingsolver. 1989. Evolution of thermal sensitivity of ectotherm performance. Trends Ecol. Evol. 4:131–135.

Huey, R. B., L. Partridge, and K. Fowler. 1991. Thermal sensitivity of *Drosophila melanogaster* responds rapidly to laboratory natural selection. Evolution 45:751–756.

Huey, R. B., and M. Slatkin. 1976. Cost and benefits of lizard thermoregulation. Q. Rev. Biol. 51: 363–384.

HUEY, R. B., AND R. D. STEVENSON. 1979. Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. Am. Zool. 19:367–384.

LENSKI, R. E., M. R. ROSE, S. C. SIMPSON, AND S. C. TADLER. 1991. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. Am. Nat. 138:1315–1341.

LEVINS, R. 1968. Evolution in Changing Environments. Princeton University Press, NJ USA.

LYNCH, M., AND W. GABRIEL. 1987. Environmental tolerance. Am. Nat. 129:283–303.

PEASE, C. M., R. LANDE, AND J. J. BULL. 1989. A model of population growth, dispersal, and evolution in a changing environment. Ecology 70:1657–1664.

PROSSER, C. L. 1986. Adaptational Biology: Molecules to Organisms. John Wiley and Sons, N.Y., USA.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry, 2nd ed. W. H. Freeman, N.Y., USA.

WALLACE, B. 1968. Topics in Population Genetics. Norton, N.Y., USA.

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