

Rapid evolution in response to high-temperature selection

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TEMPERATURE is an important environmental factor affecting all organisms^{1,2}, and there is ample evidence from comparative physiology that species^{1,3} and even conspecific populations^{4,5} can adapt genetically to different temperature regimes. But the effect of these adaptations on fitness and the rapidity of their evolution is unknown, as is the extent to which they depend on pre-existing genetic variation rather than new mutations. We have begun a study of the evolutionary adaptation of *Escherichia coli* to different temperature regimes, taking advantage of the large population sizes and short generation times in experiments on this bacterial species^{6–10}. We report significant improvement in temperature-specific fitness of lines maintained at 42 °C for 200 generations (about one month). These changes in fitness are due to selection on *de novo* mutations and show that some biological systems can evolve rapidly in response to changes in environmental factors such as temperature.

A clone of a single cell in the ancestral line maintained at 37 °C for 2,000 generations was used to found six high-temperature (42 °C) and six control (37 °C) lines. All lines (ancestral, high-temperature and control) were propagated in Davis minimal medium¹¹ supplemented with 25 µg ml⁻¹ glucose. Cultures (10 ml) were maintained by daily serial dilution into fresh medium, permitting a 100-fold increment in biomass ($\log_2 100 = 6.64$ generations of binary fission) each day. The total size of a population maintained under this culture regime cycles from $\sim 5 \times 10^6$ to $\sim 5 \times 10^8$ cells. The founding lines possessed one of two arabinose-utilization marker states^{9,10}, which were used for clonal identification in fitness assays and for monitoring possible cross-contamination between lines. Selective neutrality of these markers at both 37 °C and 42 °C was verified by preliminary experiments and no cross-contamination of lines was observed. Additional phenotypic markers were also used to exclude the possibility of contamination from external sources^{9,10}. The use of an ancestral line that was already well-adapted to the culture medium increased the likelihood that temperature-specific adaptations could be detected, as opposed to other beneficial but non-temperature-specific mutations that might enhance adaptation to the culture medium. Also, by studying spontaneous mutants that have increased in frequency gradually by selection rather than mutants obtained by mutagenesis and subsequent screening for extreme phenotypes, one avoids mutations that have profoundly deleterious pleiotropic effects and consequently would have little evolutionary significance.

After 100, 200 and 400 generations of binary fission (15, 30 and 60 days respectively), single colony isolates from each high-temperature line were made to compete against the ancestral clone possessing the alternative arabinose marker state. These competition experiments used the same culture conditions in which the high-temperature lines had been maintained. Six replicate competition experiments were run for each of the six lines at each sample time. Both competitors were preincubated at 42 °C for one day, such that each had equal opportunity to acclimatize physiologically to the culture conditions. Thereafter equal aliquots of each competitor were added to fresh medium; initial densities were determined by plating on medium that permitted the competitors to be distinguished on the basis of the arabinose-utilization marker state. The mixed culture was maintained at 42 °C for one day and the final densities of ancestral and high-temperature clones determined again by plating. These competition experiments enabled us to examine adaptive evolution (that is, improvement in fitness relative to the ancestral clone) by the high-temperature lines. Fitness (W) was defined

TABLE 1 Improvements in fitness during 400 generations at 42 °C

Generation	Estimated mean fitness over all lines*	Estimated variance in fitness among lines†
100	$\bar{X} = 0.991$ $t_s = -1.386$ $0.5 < P < 0.9$	$s_g^2 = 2.07 \times 10^{-6}$ $F_s = 1.008$ $0.25 < P < 0.5$
200	$\bar{X} = 1.085$ $t_s = 2.712$ $0.01 < P < 0.025$	$s_g^2 = 4.88 \times 10^{-3}$ $F_s = 6.122$ $P < 0.001$
400	$\bar{X} = 1.072$ $t_s = 2.827$ $0.01 < P < 0.025$	$s_g^2 = 2.92 \times 10^{-3}$ $F_s = 4.082$ $0.001 < P < 0.01$

* The mean and standard error of the mean were calculated from the means of the six fitness assays performed for each of the six lines. The corresponding t -statistic is shown, as is the significance level associated with the one-tailed hypothesis that the mean fitness across all lines relative to the ancestral clone was greater than one. The number of degrees of freedom employed in this test was five, corresponding to one less than the number of lines.

† Variance component due to differences in fitness among lines¹⁴. The corresponding F -statistic is shown, along with the significance level associated with the hypothesis that this variance was greater than zero. The corresponding degrees of freedom for the numerator and denominator in the F -ratio were 5 and 30, respectively.

as the ratio of the number of doublings for the high-temperature and ancestral clones: $W = \log_2(N_f/N_i)/\log_2(N'_f/N'_i)$ where N_i and N'_i are the initial densities and N_f and N'_f are the final densities of the ancestral and high-temperature clones, respectively^{9,10}. A value of 1 indicates that two clones have equal fitness; a value greater than 1 indicates that the high-temperature line has adapted evolutionarily.

The major results are given in Table 1. At 100 generations of culture at 42 °C there was no significant increase in fitness nor significant heterogeneity in fitness among the six lines. At 200 generations, and again at 400 generations, fitness had increased significantly relative to the ancestral clone by an average of ~ 7 –8%. Furthermore, analyses of variance indicated significant heterogeneity in fitness among the six lines at 200 generations and again at 400 generations. Thus, new mutations that increased fitness at 42 °C had occurred and reached high frequency within only 200 generations (30 days) in at least some of the high-temperature lines.

The control lines provide evidence that the adaptive evolution observed in the high-temperature lines was temperature-specific (Table 2). The six control lines maintained at 37 °C for 400 generations did not show any significant fitness improvement when competed at 37 °C against the same ancestral clone. Relative to their common ancestor, the fitness at 37 °C of the control lines was significantly less than the fitness at 42 °C of the high-temperature lines (one-tailed t -test; $t_s = 2.515$, 10 d.f., $0.01 < P < 0.025$).

TABLE 2 Temperature-specificity of improvements in fitness

Estimated mean fitness over all lines*	Estimated variance in fitness among lines†
Direct response of fitness at 37 °C after propagation at 37 °C for 400 generations	
$\bar{X} = 1.006$ $t_s = 1.164$ $0.1 < P < 0.25$	$s_g^2 = -1.28 \times 10^{-4}$ $F_s = 0.587$ $0.5 < P < 0.75$
Correlated response of fitness at 37 °C after propagation at 42 °C for 400 generations	
$\bar{X} = 1.040$ $t_s = 4.056$ $0.001 < P < 0.01$	$s_g^2 = 2.13 \times 10^{-4}$ $F_s = 1.585$ $0.1 < P < 0.25$

* See Table 1.

† See Table 1.

We also determined whether the adaptive evolution observed in the high-temperature lines produced any correlated response in fitness at 37 °C, the ancestral temperature regime (Table 2). A significant fitness advantage was observed when the high-temperature lines were competed against the ancestor at 37 °C. Although the fitness advantage observed at 37 °C (~4%) was less than the advantage observed at 42 °C (~7%), this difference was not statistically significant (one-tailed *t*-test for paired comparisons, $t_s = 0.898$, 5 d.f., $0.1 < p < 0.25$). Also, mean fitnesses at 37 °C and 42 °C were not positively correlated across the high-temperature lines (coefficient of correlation $r = -0.088$, 4 d.f., $P > 0.5$). One possible explanation for this lack of correlation across lines is heterogeneity among alleles in their effects on a correlated character^{9,12}. To examine this possibility we first computed the overall ratio of the correlated response to selection (mean change in fitness at 37 °C, Table 2) to the direct response to selection (mean change in fitness at 42 °C, Table 1): $(1.040 - 1)/(1.072 - 1) = 0.554$. This ratio provides an estimate of the regression of fitness at 37 °C on fitness at 42 °C (see equation 19.5a in ref. 13). We then used the jackknife procedure¹⁴ to determine the influence of each fitness assay on this ratio. An analysis of variance using the jackknifed data (Table 3) indicates significant heterogeneity among the lines in this ratio, and hence in the relationship between fitnesses at 37 °C and 42 °C. Five of the six lines give a positive regression coefficient (mean slope from jackknifed data = 0.782) and among these five lines there is a significant positive correlation between mean fitnesses at 37 °C and 42 °C ($r = 0.968$, 3 d.f., $P < 0.01$). The other line gives a negative regression coefficient (mean slope from jackknifed data = -0.738) that obscures the overall correlation. There is evidently heterogeneity among alleles that improve fitness at 42 °C in their effect on fitness at 37 °C.

Even with such heterogeneity, the average correlated response of fitness at 37 °C for the high-temperature lines was significantly greater than the average direct response of the control lines at 37 °C (two-tailed *t*-test, $t_s = 2.982$, 10 d.f., $0.01 < P < 0.025$). This unexpected result demonstrates that adaptation to an environment may sometimes occur more rapidly as a consequence of selection in another environment than as a direct evolutionary response. We see three possible explanations for this phenomenon in our experimental system. First, certain mutations may occur only at higher temperatures. Second, the high-temperature regime may have increased the rate of certain mutations^{6,15}, including some beneficial mutations sufficiently rare that they were not yet observed at 37 °C. Third, equivalent mutations may have occurred at the same rate at 37 °C, but were not fixed during this period because of a smaller selective advantage at this temperature (smaller selective advantage both increases the probability that a favourable mutation will be lost initially owing to genetic drift and increases the amount of time that would be required for the favoured mutant allele, if it is not lost by drift, to reach some specified high frequency^{16,17}). These results also bear on the issue of whether there necessarily exists a negative genetic correlation (that is, pleiotropic trade-off) between fitnesses measured in different environments (for example, temperatures¹⁸). The correlated improvement in fitness at 37 °C in most of the high-temperature lines clearly indicates no necessary loss of fitness in the ancestral environment (37 °C)

TABLE 3 Heterogeneity in ratio of correlated response (change in fitness at 37 °C) to direct response (change in fitness at 42 °C) among lines propagated at 42 °C for 400 generations*

Source	Degrees of freedom	Sums of squares	Mean square	F_s
Among lines	5	24.24	4.85	2.76
Within lines	66	115.91	1.76	$0.025 < P < 0.05$
Total	71	140.14		

* The overall ratio of the correlated response (Table 2) to the direct response (Table 1) is calculated in the text. The jackknife procedure¹⁴ was used to compute pseudovalues that indicate the influence of each of the 72 fitness assays (six assays at each of two temperatures for each of six lines) on this overall ratio. The grand mean of the pseudovalues was 0.529, close to the overall ratio. The analysis of variance tests for heterogeneity among lines in pseudovalues. Significant heterogeneity indicates genetic variation among lines in the effects of alleles that improve fitness at 42 °C on fitness at 37 °C. See text for details.

during adaptation to a new environment (42 °C).

Selection studies, in which one monitors the response of laboratory populations to variation in natural environmental variables, may provide a powerful tool to investigate and predict the response of natural populations to environmental variation¹⁹ (for example, climate change^{20,21}). Our experiments demonstrate that some bacteria can respond to changes in temperature in ways that improve evolutionary fitness over short absolute time periods. Mutations favouring such fitness improvement are evidently not rare and can spread rapidly through a population. Some organisms may therefore be able to track and evolve in response to temperature changes in their environments even seasonally and certainly over longer time periods. □

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