

# Temperature acclimation and competitive fitness: An experimental test of the beneficial acclimation assumption

(physiological ecology/thermotolerance/heat shock response/bacteria/*Escherichia coli*)

ARMAND M. LEROI<sup>\*†</sup>, ALBERT F. BENNETT<sup>\*</sup>, AND RICHARD E. LENSKE<sup>‡§</sup>

<sup>\*</sup>Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717; and <sup>‡</sup>Center for Microbial Ecology, Michigan State University, East Lansing, MI 48824-1325

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**ABSTRACT** Phenotypic acclimation is generally assumed to confer an advantage in the environment that stimulates the response. To test this beneficial acclimation assumption explicitly, we investigated the consequences of temperature acclimation for the fitness of *Escherichia coli* at two temperatures, 32°C and 41.5°C. Both temperatures permit growth and long-term persistence of the genotypes in serial culture. We found that prior acclimation to 32°C, relative to acclimation to 41.5°C, enhanced fitness at 32°C, consistent with the assumption. But prior acclimation to 41.5°C actually reduced fitness at 41.5°C, relative to acclimation to 32°C. Hence, the assumption that acclimation always confers an advantage is demonstrated to be false. Acclimation to 41.5°C did, however, improve survival at 50°C, a lethal temperature. This protective response has been shown to be associated with the induction of stress proteins. The reduced competitive fitness caused by acclimation at 41.5°C may reflect a physiological burden associated with expression of stress proteins when they are not needed to prevent lethal damage. Whatever the cause, acclimation to the higher temperature decreased competitive fitness at that temperature.

Organisms respond to environmental change by different mechanisms over different time scales. An individual organism may acclimate phenotypically, without any change in its genotype. Although acclimation usually occurs within an individual's lifetime, its effects may sometimes persist for several generations (e.g., ref. 1). Populations of organisms also adapt genetically by natural selection, which requires a change in genotypic frequencies. The ability to acclimate is subject to genetic influences and may itself therefore evolve. We have been performing a series of experiments to examine both short-term phenotypic acclimation to environmental temperature and long-term evolutionary modification of these acclimatory responses in the bacterium *Escherichia coli*. Here we report experiments that measure directly the fitness consequences of phenotypic acclimation to changing temperatures.

Temperature acclimation may involve alteration of many different physiological processes. Because acclimatory responses are common and frequently homeostatic (2–4), biologists have usually assumed that acclimatory responses are adaptive; i.e., that these responses benefit the organisms that manifest them (e.g., refs. 5–10). We call this the beneficial acclimation assumption. Stated another way, this assumption asserts that acclimation to a particular environment gives an organism a performance advantage in that environment over another organism that has not had the opportunity to acclimate to that particular environment. Indeed, some authors (e.g., ref. 10) restrict the definition of acclimation to phenomena having beneficial effects, although how these bene-

fits are to be judged is not stipulated. We regard the presumption of benefit to be an empirical issue that needs experimental verification.

That acclimatory responses are beneficial is not axiomatically true. Gould and Lewontin (11) argued that not all genetic differences between populations or species are adaptive and that “panglossian” explanations often ignore other plausible explanations for such differences. Similarly, not all phenotypic changes that take place within individual organisms in response to changing environments should be presumed to be beneficial, and alternative explanations must be considered. Some phenotypic changes may be inconsequential, while others may even be disadvantageous, either because they have not previously been subject to selection or because they are functionally coupled to other more important phenotypic changes that are adaptive.

The beneficial acclimation assumption has not previously been subjected to direct test by a rigorous manipulative experiment, and we sought to do that in this study. It is our view that any benefit associated with acclimation must ultimately be judged in terms of its impact on differential reproduction, or Darwinian fitness. Other presumed “benefits” to the individual organism (e.g., increased growth rate or efficiency of energy utilization) are irrelevant evolutionarily unless they enhance fitness, because the individual organism is only temporary. An assessment of the impact of phenotypic acclimation on fitness, however, is often impractical, because differential reproductive success is extremely difficult to measure for most organisms. According to the Krogh principle (12, 13), a biological phenomenon should be studied in that system in which it is most easily and rigorously examined. Here, we experimentally test the beneficial acclimation assumption, using a system in which measuring the effect of acclimatory responses on fitness is both simple and direct. With populations of bacteria, one can simply and directly measure the relative fitness of two competitors, one possessing and one lacking a trait of interest (but otherwise identical), over several generations. The two competitors share a pool of limiting resources. The rate of change in their relative abundance is used to compute their relative fitness and hence to infer the effect of the trait of interest on fitness in the environment in which the experiment is conducted. Such experiments have been performed with *E. coli* for many traits to test a variety of ecological and evolutionary hypotheses (see refs. 14–16 for reviews).

The experiments reported here are unusual insofar as the competitors are genetically identical (except for a neutral marker that permits competitors to be distinguished) but differ in their acclimation state. The traits of interest, therefore, are the sets of phenotypic attributes that are generated

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<sup>†</sup>Present address: Department of Molecular Genetics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.

<sup>§</sup>To whom reprint requests should be addressed.

by acclimation to different environments. These differences enable us to test directly the beneficial acclimation assumption. We do so by placing separate populations of a single bacterial clone into two different thermal environments: 32°C and 41.5°C. The bacteria grow in those environments for several generations, thereby allowing acclimation to these different temperatures. We then observe the growth of the marked lines in competition, so that fitness is assessed for each acclimation state in both its "own" environment (i.e., the temperature to which it has acclimated) and in the other environment (i.e., the temperature to which it has not acclimated). According to the beneficial acclimation assumption, the bacteria acclimated to each temperature should have greater reproductive success in competition at the same temperature than those acclimated to the different temperature. In a companion study (30), we examine the evolution of these acclimatory responses during long-term propagation in an environment consisting of a daily alternation between these two temperatures.

## MATERIALS AND METHODS

**Bacterial Strains.** These experiments were conducted with a genotype of *E. coli* B isolated from a population that had been maintained for 2000 generations at 37°C (17). During this time, the population was propagated by serial dilution in a glucose-limited minimal medium, with  $\approx 6.7$  cell generations per day. This genotype, designated REL1206, is unable to utilize arabinose for growth (Ara<sup>-</sup>), but an Ara<sup>+</sup> mutant (REL1207) was obtained by plating cells on minimal arabinose medium (18). These two variants, Ara<sup>+</sup> and Ara<sup>-</sup>, are readily distinguished by colony coloration on tetrazolium/arabinose (TA) indicator agar, allowing their abundances to be enumerated simultaneously in competition experiments (19). The effective neutrality of the marker state in the glucose-limited minimal medium was verified at the temperatures used in these experiments (ref. 18; see Table 1, rows 1 and 2). These marked clones also served as the founding genotypes for a separate experiment on genetic adaptation to different thermal regimes (18, 20–22).

**Medium and Culture Conditions.** Experiments were performed in DM medium (23) supplemented with 0.002  $\mu$ g of thiamin hydrochloride and 25  $\mu$ g of glucose per ml. Cultures consisted of 10 ml of medium in 50-ml Erlenmeyer flasks, placed in shaking incubators (120 rpm) at the indicated temperatures. These conditions were identical, but for temperature, to the historical environment of the genotype used in this study (see above).

**Fitness Assays and Definition.** Fitness assays consisted of two sequential steps, acclimation and competition. In the acclimation step, two bacterial populations were separately grown for 24 hr in DM medium at either 32.0°C or 41.5°C ( $\pm 0.5^\circ\text{C}$ ). During this step, the bacteria underwent several cell generations and, in so doing, exhausted the limiting glucose. The bacteria also may have undergone phenotypic changes caused by their exposure to one temperature or the other; these acclimatory responses may have occurred during growth, stationary phase, or both. In the competition step, aliquots of the two separately acclimated and reciprocally marked bacterial populations were simultaneously diluted (1:200 each) into a common flask of fresh DM medium, whereupon an initial sample was plated on TA indicator agar. This mixed population was then incubated for 24 hr at either 32°C or 41.5°C, at which time a final sample was plated on TA agar. During the competition step, the mixed population also reached stationary phase after exhausting the available glucose.

In another experiment, the acclimation and competition steps were carried out in a series of flasks placed along a thermal gradient from 41.3°C to 42.2°C ( $\pm 0.2^\circ\text{C}$ ), which was

stably maintained in a shaking incubator (21). Previous results indicated that fitness measurements involving the genotype used in this study are quite sensitive to even slight differences in temperature in this range (21).

The relative fitness,  $W$ , of the differentially acclimated (and reciprocally marked) populations can be calculated from the change in their relative abundances between the initial and final samples in the competition step:

$$W = \log_e(N_{42}^f/N_{42}^i)/\log_e(N_{32}^f/N_{32}^i),$$

where  $N$  indicates population density, subscripts 32 and 42 denote acclimation to lower and higher temperatures, respectively, and superscripts  $i$  and  $f$  indicate initial and final samples, respectively. Thus defined,  $W$  is equivalent to a ratio of the realized Malthusian parameters of the two populations during competition with one another in the experimental environment (17). A value of  $W = 1$  indicates that the two competitors had equal fitness. Unequal fitness may reflect a difference in survival and/or reproductive success during any phase of the competition step (i.e., lag, growth, and stationary phases).

Note that we express fitness of the population acclimated to 41–42°C relative to the population acclimated to 32°C, irrespective of whether the competition step is at 32°C or the higher temperature. Therefore, according to the beneficial acclimation assumption, we expect  $W < 1$  when competition takes place at 32°C and  $W > 1$  when competition is at 41–42°C.

**Thermotolerance.** To examine differential thermotolerance conferred by acclimation, death rates for bacteria acclimated to 32°C and 41.5°C were measured at a high temperature lethal to this clone (21). Following the same protocol used for the acclimation step of the fitness assay, stationary-phase bacterial populations were transferred directly into a water bath maintained at  $50 \pm 1.0^\circ\text{C}$ . Samples from these populations were obtained after 0, 15, 30, 60, 90, 120, 180, and 240 min and plated to determine the number of viable bacteria (colony-forming units). The natural logarithm of this number was regressed against time spent at 50°C to calculate the death rate of the population.

## RESULTS

During competition at 32°C, bacteria that were acclimated to 32°C had a clear fitness advantage relative to those that were acclimated to 41.5°C, irrespective of the marker state of the clones acclimated to their respective temperatures (Table 1, rows 3 and 4). This result therefore accords well with the

Table 1. Effect of acclimation temperature on competitive fitness at 32°C and 41.5°C

Acclimation temperature,* °C		Competition temperature, °C	$n$	Fitness		
Ara <sup>+</sup>	Ara <sup>-</sup>			Mean	SE	$P^\dagger$
32	32	32	10	1.006 <sup>‡</sup>	0.013	0.629
42	42	42	10	0.993 <sup>‡</sup>	0.012	0.588
32	41.5	32	15	0.938 <sup>§</sup>	0.012	<0.001
41.5	32	32	15	0.903 <sup>§</sup>	0.011	<0.001
32	41.5	41.5	15	0.840 <sup>§</sup>	0.012	<0.001
41.5	32	41.5	15	0.813 <sup>§</sup>	0.012	<0.001

\*Ara<sup>+</sup> and Ara<sup>-</sup> refer to genotypic variants with alternative arabinose-utilization marker states.

<sup>†</sup>Two-tailed probability of rejecting the null hypothesis that mean fitness equals 1, based on  $t$  test with  $n - 1$  degrees of freedom.

<sup>‡</sup>Mean fitness of Ara<sup>+</sup> relative to Ara<sup>-</sup> (data from ref. 18).

<sup>§</sup>Mean fitness of the 41.5°C-acclimated variant relative to the 32°C-acclimated variant.

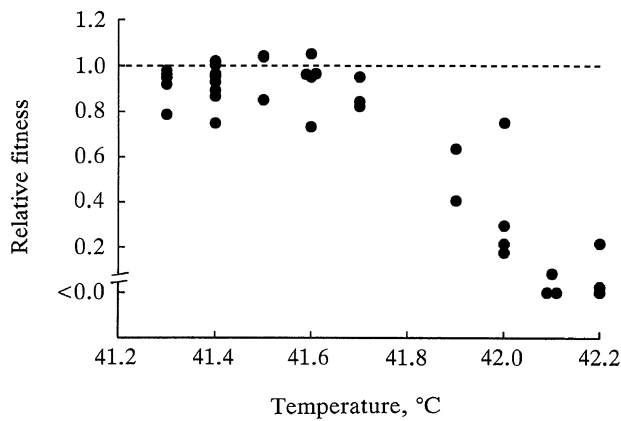


FIG. 1. Effect on competitive fitness of acclimation to high temperatures relative to acclimation at 32°C. The experimental temperature was applied during the acclimation step (to one competitor only) as well as during the competition step of the fitness assay. A relative fitness ( $W$ ) < 1 indicates that prior acclimation to the high temperature had a deleterious effect on fitness at that temperature, relative to acclimation at 32°C.

beneficial acclimation assumption.

According to the beneficial acclimation assumption, it is also expected that bacteria acclimated to 41.5°C will be more fit than bacteria acclimated to 32°C (i.e.,  $W > 1$ ), when competition takes place at 41.5°C. In fact, the opposite result was obtained (Table 1, rows 5 and 6): the 32°C-acclimated cells were significantly more fit than their 41.5°C-acclimated counterparts in competition at 41.5°C. Again, the result is independent of the marker state of the differentially acclimated competitors. Therefore, acclimation to high temperature actually reduced competitive fitness at high temperature, contrary to the beneficial acclimation assumption.

To examine the effect of high-temperature acclimation on competitive fitness in more detail, we measured the fitness consequences of acclimation and subsequent competition along a thermal gradient from 41.3°C to 42.2°C, relative to a competitor conditioned at 32°C (Fig. 1); 42.2°C is at the upper limit for population persistence of this genotype in the serial culture regime used in this study (21). Between 41.3°C and 41.7°C, acclimation to these temperatures was moderately disadvantageous relative to acclimation at 32°C (mean  $W = 0.924$ , SE = 0.018,  $P < 0.001$ , based on two-tailed  $t$  test with

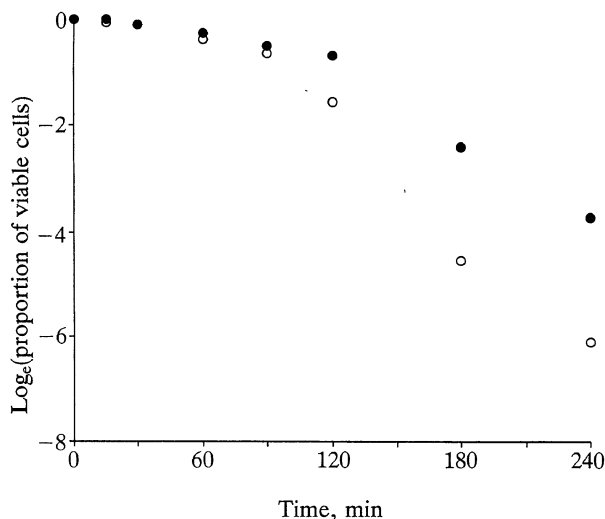


FIG. 2. Effect of acclimation temperature on death rate at a lethal temperature, 50°C. Open circles represent cells acclimated at 32°C; filled circles represent cells acclimated at 41.5°C. Each point represents the mean of six replicate assays; for statistical analysis, see Table 2.

Table 2. Effect of acclimation temperature (32°C or 41.5°C) on death rates at lethal high temperature

Period at 50°C during which death rate was measured, min	Death rate at 50°C, min <sup>-1</sup>			
	32°C	41.5°C	$t$	$P$
0–240	0.027	0.016	11.60	<0.001
0–60	0.006	0.005	1.42	0.931
90–240	0.038	0.023	10.32	<0.001

Death rates were calculated by linear regression of  $\log_e$ (proportion of viable cells) versus time. Regressions were performed separately for each replicate using all eight time points, and then using only the first four or last four points. Six independent replicates were performed at each assay temperature.  $P$  values are one-tailed probabilities of rejecting the null hypothesis of no effect of acclimation temperature, based on  $t$  test with  $n_1 + n_2 - 2 = 10$  df.

$n - 1 = 23$  df), consistent with the results in Table 1. Between 41.9°C and 42.2°C, the deleterious effects of acclimation to high temperature became even more severe, so that in some replicates, the high-temperature-acclimated population had not even begun to grow by the time that the 32°C-acclimated cells consumed the limiting glucose (i.e.,  $W \approx 0$ ). Evidently, the deleterious effect of high-temperature acclimation on competitive fitness has a strong thermal dependence. However, it is not caused by the lethality of the high temperature *per se*, since the genotype used in these experiments can sustain itself indefinitely by sufficient growth to offset serial dilution and death even at these higher temperatures (21).

Although acclimation to 41.5°C unexpectedly reduced competitive fitness at that temperature, such acclimation did confer thermotolerance to even more extreme temperatures. The 41.5°C-acclimated bacteria had significantly lower death rates when subsequently exposed to 50°C than did bacteria acclimated to 32°C (Fig. 2 and Table 2). Indeed, a reduction in death rate at a lethally high temperature following exposure to a high but permissive temperature fulfills the operational definition of the heat shock response (24, 25). It is therefore clear that some physiological adjustments to high temperature did occur during the acclimatory phase at 41.5°C, even though these adjustments did not improve competitive fitness at that temperature.

## DISCUSSION

The beneficial acclimation assumption predicts that bacteria acclimated to a particular temperature should be significantly more fit at that temperature than bacteria acclimated to some other temperature. One of our two reciprocal acclimation and competition experiments gave that result: 32°C-acclimated bacteria were more fit than 41.5°C-acclimated bacteria in subsequent competition at 32°C. However, the 32°C-acclimated bacteria also were more fit than the 41.5°C-acclimated bacteria at 41.5°C, contrary to the beneficial acclimation assumption.

We must therefore reject the generality of the beneficial acclimation assumption: a fitness benefit may or may not result from acclimation. In fact, fitness in a particular environment may actually decrease because of acclimation to that environment. A single negative example is sufficient to falsify the generality of a hypothesis, and we found such an exception in the first explicit test of this one.

How can we be sure that these effects—both beneficial and adverse—are caused by phenotypic acclimation rather than genotypic adaptation? We have previously studied the genetic adaptation of this same bacterial strain to these same thermal regimes (18, 20). At 32°C, no significant genetic adaptation was observed for >400 generations (60 days), whereas the beneficial acclimatory effect reported here is manifest in 6–7 generations (1 day). At 41.5°C, we observed

genetic adaptation within 200 generations (30 days), but not within 100 generations. Again, the acclimatory effect of 41.5°C is manifest in only 1 day, and it has the opposite effect of genetic adaptation, leading to a decrement in performance. The fitness differences reported here are due to phenotypic acclimation rather than genetic adaptation.

How are the fitness effects associated with acclimation manifest during competition? The serial dilution regime used in this study encompasses a series of more or less distinct phases of population growth. A competition experiment is begun by placing two competitors, taken from stationary-phase cultures, into fresh medium. The bacteria begin a period of metabolic activation and synthesis prior to the commencement of cell division (lag phase). They then enter a period of rapid cell division (growth phase). Eventually the bacteria exhaust the limiting supply of glucose so that they cannot grow and may experience some death (stationary phase). The transitions between these phases need not be sharp: for example, populations may comprise both growing and nongrowing cells during the early or late phases of growth. Higher relative fitness in a competition experiment must be mediated through better performance in one or more of these phases of population growth dynamics. That is, a shorter lag phase, a higher rate of growth, or reduced mortality during starvation will, alone or in combination, increase reproductive success and hence competitive fitness.

From an evolutionary perspective, it is immaterial which of these phases is the locus of differential performance. Such differences may have straightforward physiological or biochemical explanations, but their simplicity does not detract from their profound fitness effect. We have not examined which population phases are responsible for the fitness differences observed in these experiments. Had we performed similar experiments using bacterial populations in growth phase only, the beneficial acclimation assumption might have been rejected simply because bacteria acclimated to 32°C continued to grow at the rate characteristic of that temperature for some time after they were shifted to the higher temperature. However, the unexpected disadvantage of acclimation to high temperature cannot be caused by continuation of more rapid growth by the population acclimated to 32°C, because neither population was growing at the start of the competition step (each having reached stationary phase during its separate acclimation treatment). We suspect that a shorter lag phase for the 32°C-acclimated bacteria was at least partly responsible for their greater fitness at both temperatures, because an acclimatory response to a previous environment will eventually wear off in a new environment. However, we cannot exclude the possibility that the acclimation treatments also influence subsequent growth rate or even survival in stationary phase.

How can the observed loss of fitness caused by acclimation to high temperature be reconciled with the many studies of bacteria as well as eukaryotic species (5–10, 24–26) that have reported improved functional capacities resulting from high-temperature acclimation? In these other studies, the functional capacities usually considered are not growth rate or fitness at the permissive high temperatures that induce the acclimatory response, but rather death rate under more extreme and typically lethal conditions (e.g., increased critical thermal maxima). We also observed a reduction in death rate, measured at a lethally high temperature, following acclimation of the bacteria to a permissive high temperature (Fig. 2 and Table 2), and hence our results are in fact concordant with these other studies.

It is possible that both the loss of competitive fitness at 41.5°C and the improved survival at 50°C of the 41.5°C-acclimated bacteria are consequences of a single physiological process. One candidate is the heat shock response, which in *E. coli* entails the induction of an assemblage of stress

proteins at high, but sublethal, temperatures (24–29). The expression of these proteins is associated with reduced mortality at lethal temperatures, although the precise causal relationship between these phenomena is unclear (25, 26, 28, 29). Expression of at least some heat shock proteins is an increasing function of temperature in *E. coli*, and 42°C is sufficient for their induction (24, 25). The competitive disadvantage of cells acclimated to 41.5°C and slightly higher temperatures might therefore reflect the physiological burden associated with the elevated expression of stress proteins when they are not actually needed for protection against lethal temperature. But heat shock proteins are not the only plausible explanation for the decreased fitness of the 41.5°C-acclimated group. Another possibility, for instance, is the more rapid degradation of some growth-essential protein during stationary phase at higher temperatures, which could extend the subsequent lag phase upon transfer to fresh medium at both low and high temperatures. However, induction of the heat shock response could simultaneously account for both the fitness decrement at 41–42°C and the improved thermotolerance at 50°C.

Whatever the precise physiological basis of these effects, the bacteria clearly undergo significant phenotypic changes during acclimation to high temperatures. However, these phenotypic changes are beneficial only in other, even hotter, environments, and they are actually disadvantageous at the growth-permissive temperatures that stimulate them. In this case, acclimation seems to take the form of hedging a bet that the environment will continue to become even hotter, rather than remaining at a high but tolerable temperature. Perhaps defense against further temperature increments has been more important in the evolutionary history of these bacteria than maintaining competitive ability in stressful but nonlethal temperatures. In this regard, it is interesting that thermotolerance (i.e., improved survival upon exposure to 50°C) declines significantly when these bacteria are serially propagated at 41.5°C for up to 14 days (A. Cullum, A.F.B., and R.E.L., unpublished data). Such a trend suggests that the heat shock response is turned down when a high but growth-permissive temperature is sustained, rather than being followed by a lethally high temperature.

Although 32°C and 42°C are symmetric around the temperature (37°C) at which these bacteria had been propagated for the preceding 2000 generations, there are several objective criteria by which 42°C may be considered the more stressful temperature (22). (i) Relative to 37°C, 42°C reduces both maximal growth rate and yield, whereas 32°C reduces only the former. (ii) Forty-two degrees Celsius is within  $\approx 0.5^\circ\text{C}$  of the critical thermal limit for extinction of the bacterial population under our standard culture conditions, whereas 32°C is  $>10^\circ\text{C}$  away from both the upper and lower thermal limits. (iii) Whereas 42°C induces expression of stress proteins that mediate a protective response, 32°C does not. Our *a priori* expectation was that acclimation to a stressful environment would be more important than acclimation to a nonstressful environment for competitive fitness in the respective environments. However, the opposite result was obtained. Whether this unexpected result is itself generalizable remains to be seen. But in light of our results and the possible explanations for them, it now seems plausible to us that acclimation to a stressful but nonlethal environment may often be disadvantageous to the organism in that environment. That is, induction of a protective response to a lethal environment may require that an organism be reasonably vigorous, and so, such responses may need to be induced before conditions become too damaging. However, there are physiological costs associated with the induction and expression of the stress response, and these costs may translate into reduced reproductive success if the environment does not progress to the damaging state. Under this

interpretation, the acclimatory response of the bacteria to high temperature would in fact be beneficial in certain sequences of environments, even if not the one used in our experiments. This possibility raises interesting questions about the sequences of thermal environments that *E. coli* typically experiences in nature.

It is important to emphasize that although 41.5°C is stressful to the bacterial genotype used in these experiments, it is not lethal: in fact, one can maintain growing and viable populations indefinitely at temperatures up to about 42.2°C (21, 22). That is, fitness does not steadily decrease over time and populations do not eventually go extinct at this temperature. Therefore, the depression in competitive fitness caused by acclimation to high temperature is not simply an inconsequential character that is correlated with lethality, such as a lobster turning red when placed in boiling water.

We do not know whether our results are specific to these temperatures and this particular bacterial genotype or whether similar results might be obtained for the effects of thermal acclimation with other genotypes and species and even for acclimation to other types of environmental change. Only a mixture of further experimental and comparative studies can reveal the breadth of applicability of these observations. We have, however, disproved the generality of the beneficial acclimation assumption. Whatever the precise mechanistic basis, our results demonstrate that phenotypic changes arising during acclimation to a particular environment cannot automatically be assumed to increase fitness in that same environment. Moreover, the phenotypic changes that occur during acclimation may have dramatically different fitness consequences depending on which components of fitness are assayed and in what environment the assay is performed.

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