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Experimental investigations of evolutionary adaptation to temperature

Comparative and experimental studies of adaptation

Comparative and experimental analysis

Organismal biologists employ two principal methods in their investigation of the natural world: comparison and experiment. The latter is most familiar in the context of laboratory investigations of functional mechanisms. Experiment is the classic application of the scientific method, including such elements as rigorous and replicated design, controlled manipulation of a single variable of interest and the incorporation of a control group into the study. While experimental science has been crucial to our understanding of how organisms work, to date it has had relatively less application in studying how those organisms came to be the way they are, i.e. in studies of the evolution of organismal characters. In such evolutionary studies, comparative investigations have been by far the dominant methodological tradition.

In the study of evolutionary adaptation to temperature, for example, virtually all our knowledge is derived from comparative studies of different populations, species, or other taxa inhabiting different thermal environments (for reviews, see Precht *et al.*, 1973; Prosser, 1973; Hochachka & Somero, 1984; Cossins & Bowler, 1987). The comparative approach involves the measurement of a character and its correlation with environmental temperature. If the character (e.g. a rate process) is thermally dependent, then it is measured either over a similar range of temperatures or at a single temperature common to the different groups examined. The pattern of character on environmental temperature is then analysed and interpreted, most frequently in an adaptive context (Prosser, 1986; Cossins & Bowler, 1987; Bennett, 1996), and compared with the pattern found for other biological systems inhabiting similar thermal environments. Applications of this approach include, for example, the classical studies of thermal adaptation in different populations of killifish (Powers, 1987), species of barracuda (Graves &

Somero, 1982), and groups of tropical, temperate, and arctic ectotherms (Scholander *et al.*, 1953). These and other such studies have been important in documenting the fit between functional capacity and thermal environment and the associated adaptive shifts in rate processes and thermal niche. Over the past decade, the comparative methodology has incorporated an historical analytical component (Felsenstein, 1985; Brooks & McLennan, 1991; Harvey & Pagel, 1991). Phylogenetically-based comparative studies have the additional advantage of being able to falsify adaptive hypotheses, as well as reconstruct the putative ancestral condition and calculate rates of evolution (Feder, 1987; Huey, 1987; Garland & Adolph, 1994; Bennett, 1996). Examples of such studies examining evolutionary adaptation to temperature include analyses of burst escape speed of lizards (Huey & Bennett, 1987), activity metabolism of anurans (Walton, 1993), and thermoregulation in fish (Block *et al.*, 1993).

Comparative evolutionary analyses, however, have practical limitations and interpretive shortcomings even when they are phylogenetically based (Lauder *et al.*, 1993; LeRoi *et al.*, 1994*b*). For example, they depend crucially on the availability of an independently-derived set of historical relationships and the accessibility of organisms from the groups contained therein. Phylogenies are only tentative hypotheses of relationships, subject to revision (Felsenstein, 1985; Harvey & Pagel, 1991). Conclusions of comparative studies based on phylogenies are consequently also subject to revision, even though the functional observations and measurements of each group remain unaltered (for an example, see the revision of the conclusions of Huey & Bennett, 1987, by Garland *et al.*, 1991, based on newer information concerning branch lengths within the phylogeny). Comparative adaptive studies must, therefore, be tentative, dependent on potential revision of their phylogenetic bases. Furthermore, comparative analytical methodologies all must assume, in one form or another, the principle of parsimony or Occam's razor (Brooks & McLennan, 1991; Harvey & Pagel, 1991; Martins & Garland, 1991): the accepted evolutionary pattern is the one that involves the fewest character transitions. This is a necessary assumption for discrimination among different potential patterns, but it must be recognised that it is only an assumption about how evolution occurs or has occurred. The evolution of any particular character of interest may not have been parsimonious. The intermediate stages of the evolutionary diversification are not observable, so there is no way of knowing for certain whether a parsimony-based analysis is a correct historical description. Comparative evolutionary analyses must therefore remain *ex post facto* interpretations of unobserved events.

Direct experiments studying evolutionary diversification would seem to be a valuable supplement because of the limitations of comparative analyses for studying adaptation. Experimentation could provide exactly those elements lacking in comparative analyses, namely control of the selective environment, direct observation of the ancestral and intermediate (in addition to terminal) stages, and experimental controls and replication. At first consideration, evolutionary experiments might be thought to be impossible, in spite of their desirability. Locating multiple populations, each of large size, and altering the environment of some while leaving others as controls is a daunting experimental challenge. Moreover, the long generation times of most organisms commonly studied by comparative physiologists preclude direct observations of evolutionary change. Some types of organisms, however, are in fact almost ideal for such studies. The genius of comparative physiology has been the recognition and pursuit of the proper type of organism to investigate the question at hand in an expeditious manner (Krogh, 1929; Krebs, 1975). Direct experiments on evolutionary adaptation are in fact quite feasible. It is simply a matter of choosing the best organism for the study.

Experimental evolution

The essence of any experiment is design and control, and experimental studies of evolution are no different in this respect. They require the design and creation of a new series of populations and the subsequent monitoring of genetically-determined phenotypic change in those populations. Experimental evolution involves the imposition of a novel environment on replicated experimental populations, while maintaining control populations for comparison. The control populations permit a statistical evaluation of phenotypic changes attributable to the experimental manipulation, as opposed to random or directional changes in response to factors other than the experimental manipulation. For instance, a character such as body length may be increasing in the experimental populations. Observations on control groups maintained in the original environment permit a determination of whether similar changes are occurring in those populations as well, and may therefore be part of a continuing evolutionary response to some aspect of the environment other than that which was explicitly manipulated. Replication of experimental populations also permits a determination of the directionality of the evolutionary response in contrast to chance divergence (Travisano *et al.*, 1995). Effectively, replication of experimental lines is equivalent to replaying the tape of life, as proposed by Gould

(1989), to evaluate the inevitability of any particular evolutionary change in terms of its direction or underlying mechanism. Finally, in contrast to comparative studies, which are able to observe only evolutionary products, experimental evolutionary studies do not require extrapolation because they can directly measure characters in their initial, intermediate and derived conditions.

Evolution, by definition, involves intergenerational genetic change. Experimental evolutionary studies require the use of organisms with generation times that are relatively short in comparison to the duration of the experiment. Through either selection on variation existing in the original population or selection on new variation arising through recombination or mutation, genetic change is more likely, and is more likely to be detected, with greater numbers of generations. In addition to monitoring the direction of evolutionary change, multi-generational data permit determination of the rate and form of evolutionary change, as well as its trajectory. Experimental evolutionary studies may additionally require that the size of each component population be large, ideally thousands or millions of organisms in each population, to avoid founder effects and genetic drift. Investigators have utilised a variety of laboratory-cultured organisms, including fruit flies, yeast, protists and bacteria (see later) because of these requirements. These organisms can be maintained in discrete populations of very large size in defined and carefully regulated environments. In some of these organisms, measurement of competitive (Darwinian) fitness is also possible, so that the magnitude and rate of adaptation can be determined directly as change in relative fitness.

It is important to recognise that other types of evolutionary studies, while interesting and important, differ fundamentally from experimental evolution. For example, descriptions of the operation of natural selection in the wild (for examples, see Endler, 1986) are not experimental evolution, unless some experimental modification has been introduced, with appropriate replication and controls, into the system examined. Additionally, experimental evolution is not artificial selection. Artificial selection has one or more predefined criteria for the product of the evolutionary process (e.g. a morphological trait). Only organisms that meet the criteria are permitted to remain in the breeding population, and the selective criteria are continually altered to produce organisms with the desired traits. In contrast, experimental evolutionary studies create selective environments and then observe evolutionary change, in whatever form it may take. The approach has consequently been termed 'natural selection in the laboratory' (Rose *et al.*, 1990), although it may also be undertaken in the field [see, for example, the transplant

experiments of Reznick and coworkers altering predatory environments of natural populations of guppies (Reznick & Bryga, 1987; Reznick *et al.*, 1990)].

Experimental studies of evolutionary adaptation to temperature

Experimental evolutionary investigations have frequently utilised temperature as the manipulated environmental variable and examined the adaptation of their subject populations to novel thermal regimes. Temperature, and change in temperature, for the ectothermic organisms in these studies is, of course, both environmentally relevant and biologically significant, particularly in view of interest in evolutionary responses to climate change. Even theoretical models of evolution (e.g. Levins, 1968; Lynch & Gabriel, 1987; Pease *et al.*, 1989) frequently use temperature change as an analytical example because of its manifest biological importance and illustrative potential.

The first experimental study of evolutionary adaptation to temperature was undertaken over a century ago by the Rev. W. H. Dallinger (1887). Dallinger, in an attempt to demonstrate that organisms can adapt to environmental change, slowly increased culture temperature for three different species of flagellate protozoans. He was successful in greatly increasing maximal temperatures tolerated in his cultures (from 22 to 70 °C) over a period of several years. He found long periods when no adaptive progress was made, punctuated by times of very rapid improvement in thermal tolerance (perhaps the appearance and selection of favourable mutations), and noted a trade-off in growth at high and low temperatures of the adapted lines. Although his experiments lacked certain features, such as thermal and contamination controls, they were extraordinary for their time. In a letter to Dallinger, Darwin declared that he found them to be 'extremely curious and valuable' and the adaptive responses of the protists to be 'very remarkable' (Dallinger, 1887).

More recent investigations of experimental evolution in different thermal environments have utilised a variety of organisms. Fruit flies (*Drosophila* spp.) have been particularly popular as experimental subjects because of the ease of their long-term laboratory culture, the obvious impact of temperature on their functional capacities, and the wealth of information about their genetics and other aspects of their biology. Several laboratories have now maintained replicated populations of flies at different temperatures for over a hundred generations and have examined the evolution of such characters as heat tolerance,

body size, and shift in thermal niche (for examples, see Cavicchi *et al.*, 1989; Huey *et al.*, 1991; Partridge *et al.*, 1995). In addition, similar experiments are feasible using many other types of eukaryotic organisms, including nematodes (Grewal *et al.*, 1994), protists (Walton *et al.*, 1995), and fungi (Jinks & Connolly, 1973). The use and further development of these and other model systems for experimental evolutionary studies is certain to occur in the near future. In our own studies of evolutionary thermal adaptation, we have chosen to use bacteria as experimental subjects. We review below the utility of bacteria for these types of studies, the structure of our experiments and conclusions, and their implications for evolutionary adaptation to temperature.

Experimental studies of temperature adaptation in bacteria

Bacteria as experimental organisms

Bacteria possess all of the characteristics mentioned in the foregoing discussion of desirable attributes for subjects of experimental evolutionary studies. In addition, some bacteriological techniques are particularly useful for such studies. For example, bacterial populations can be preserved in a frozen state and resuscitated later for analysis. This means that direct comparisons can be made between the ancestral and derived genotypes under identical environmental conditions. Bacteria are also easily cloned, allowing the experimenter to found replicate populations with initially identical genetic composition. Finally, the wealth of information on the biochemistry, molecular biology and genetics of certain bacterial species, most notably *Escherichia coli*, may allow one to dissect measurable fitness changes into their underlying physiological adaptations and elucidate the genetic mechanisms that operate under a natural selection regime.

Given the practical advantages that these organisms offer, we chose to use bacterial populations to study thermal adaptation and to address some general questions regarding the process of evolutionary adaptation of organisms to the environment. How rapid is the evolutionary response to a change in temperature? How specific is the adaptive response to a particular environmental change? Does the direction or magnitude of change in temperature influence the dynamics of the evolutionary response? Does adaptation to a novel thermal environment imply trade-offs in performance in the ancestral environment or at other temperatures? Will replicate populations diverge as a result of finding unique solutions to identical environmental conditions, or will

they repeatedly arrive at the same physiological adaptations to a common environment?

In the following sections, we review the structure of our experiments, their outcomes, and their implications for adaptive evolution. Detailed methods and analyses can be found in the original papers (Bennett *et al.*, 1990, 1992; Lenski & Bennett, 1993; LeRoi *et al.*, 1994*a*; Travisano *et al.*, 1995; Bennett & Lenski, 1996; Mongold *et al.*, 1996).

Overview of the experimental system

In our studies of evolutionary adaptation to temperature, we utilised a lineage of *Escherichia coli* B that lacks both plasmids and bacteriophage and is therefore strictly asexual. Thus we were able to establish a series of genetically identical, asexual populations all derived from a common ancestor, place them in different thermal environments and observe the adaptive responses and consequences which arose through de novo mutation and natural selection. The large population sizes and replication of populations maintained in each experimental environment make genetic drift an unlikely explanation for any group effects which may be observed. Immigration of naturally-occurring *E. coli* into our populations was ruled out by monitoring a series of genetic markers which are not typically found in wild strains. Migration between experimental populations was also monitored and ruled out by introducing a neutral genetic marker into the ancestral genotype and interspersing replicate populations with alternate states of this marker.

At the beginning of the experiment, and at subsequent intervals, samples from the evolving populations were removed and stored at −80 °C. These samples can be stored indefinitely and subsamples can then be thawed and used in future analyses. This feature of bacterial cells means that direct comparisons of the ancestral and derived genotypes may be conducted under identical environmental conditions. This is an extremely important factor in the experimental design because it allows heritable, genetic changes to be distinguished from physiological responses as a result of acclimation to different environments.

Our experimental populations were maintained and evolved in serial transfer culture. Propagation involved transferring a fraction of each population to fresh media on a daily basis. After an initial lag phase, the populations grow exponentially until the limiting nutrient (glucose in our experiments) is depleted and growth ceases. The bacteria then enter a quiescent stationary phase, which is maintained until new nutrients become available. This 'feast or famine' availability of resources and consequent cycling of growth phase may more closely

approximate the condition of bacteria in nature than the continuous growth and stable (but low) resource levels provided in chemostats. Additionally, the multiple population growth phases afforded by serial transfer culture may allow a greater number of potential adaptive mechanisms in novel thermal environments. The length of the cycle period and size of the fraction transferred simply represent a constant level of random mortality in the environment. Although the growth rate differs with temperature, the experimental populations are limited by resource availability and, therefore, undergo the same number of generations per cycle in all of the thermal environments.

We used two experimental measurements of fitness to assess the pattern of adaptation in our experimental populations. The first is the Malthusian parameter, or absolute fitness (Bennett & Lenski, 1993), of a particular genotype in a particular environment. The absolute fitness is defined as the slope of the natural logarithm of population density regressed over time. This may be estimated for each genotype in pure culture under identical environmental conditions at varying temperatures. This provides an operational definition of the bacterium's thermal niche. The thermal niche encompasses the range of temperatures over which a genotype can grow fast enough to maintain a constant population size. To persist in our serial transfer environment, a population must be capable of increasing its population size 100-fold within a 24-h period. Outside of its thermal niche, a genotype would be incapable of maintaining a constant population size in the face of daily serial dilution, and its density would decline over time leading to eventual extinction. A negative Malthusian parameter therefore indicates that the population is growing more slowly than the dilution rate imposed by the environment.

The second measurement is the Darwinian fitness of a derived genotype relative to its ancestor. This quantity provides a measurement of a particular genotype's competitiveness in the environment of interest and, from an evolutionary point of view, is the most important property of an organism. Relative fitness is defined as the ratio of the Malthusian parameters of an evolved line and its ancestor under conditions of direct competition (Lenski *et al.*, 1991). Populations of the lines that are to compete are first separately acclimated to the environment of interest. After this preconditioning, the populations are mixed in fresh media and their relative abundances determined at the beginning and end of a specified time interval. An easily scorable genetic marker that is neutral in the assay environment is used to differentiate the two competitors in mixed culture. The rate of increase, or Malthusian parameters, for each competitor can then be calculated and relative

fitness obtained from their ratio. By making replicated measures of relative fitness in a controlled environment, one can calculate the measurement error inherent in the experimental procedure and determine the significance of even small differences in the mean fitness of a population as it changes through time. Similarly, the ability to found replicate populations with genetically identical individuals means that the genetic variation measured among populations can be partitioned into variation as a result of: (i) chance differences in the beneficial mutations that have arisen in populations maintained under identical conditions; (ii) differences among treatment groups experiencing different environments; and (iii) genotype by environment interactions. This type of analysis is the same as the approach taken in comparative studies of adaptations among phylogenetically related taxa inhabiting similar and dissimilar habitats. The difference is that in laboratory evolution experiments, the phylogenetic relationships of the populations and their environmental histories are completely known and under the control of the experimenter. The phylogeny and thermal history of the experimental lineages in our study are illustrated in Fig. 1.

Evolutionary responses to novel thermal environments

In the experimental study outlined in Fig. 1, 30 populations were founded as clones from a single ancestral genotype. This ancestor was the product of a lineage of *E. coli* B that had been maintained in the laboratory under constant environmental conditions (serial transfer culture in minimal glucose medium at 37 °C) for 2000 generations (Lenski *et al.*, 1991). During this period, the lineage underwent extensive genetic adaptation to the laboratory culture conditions, but experienced relatively little further adaptive change during subsequent culture (Lenski & Travisano, 1994).

The 30 new experimental populations were divided into five treatment groups with six replicate lines each. These five groups were propagated for an additional 2000 generations under similar culture conditions, the only difference being the temperature at which the populations were incubated (20, 32, 37 and 42 °C, and daily alternation between 32 and 42 °C). The groups are named according to the temperature at which they were propagated, as indicated in Fig. 1. Using an ancestor from a lineage already well adapted to the culture environment was presumed to increase the likelihood that any further adaptation would be a specific response to a novel thermal environment rather than further adaptation to the general culture conditions. The 37 °C group served as a control to estimate the extent of further adaptation to general

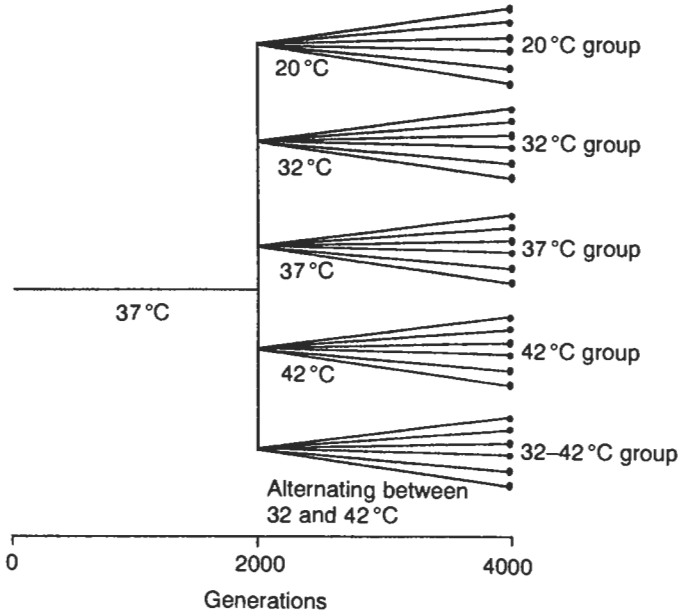

Fig. 1 Phylogeny and thermal history of the experimental lines and groups being described (derived from Mongold *et al.*, 1996.) Culture temperatures are shown below the designated lines, and group names are given at the right.

culture conditions because 37 °C was the ancestral temperature. An equal increase and decrease of 5 °C from the ancestral temperature were experienced by the 42 and 32 °C groups, respectively; however, 42 °C is also within 1 °C of the upper limit of the ancestral thermal niche, above which the ancestor cannot maintain itself in serial dilution culture. The corresponding lower thermal niche boundary of the ancestor is just below 20 °C, very close to that experienced by the 20 °C group. These four experimental groups were exposed to constant thermal environments, while the 32–42 °C group experienced and evolved in a variable thermal environment.

The relative fitness of clones isolated from each experimental line was measured relative to the common ancestor of all thirty lines (Fig. 2). The measurements of relative fitness were conducted at the temperature at which each group had evolved. For the 32–42 °C group, relative fitness was separately measured at 32 and 42 °C to assess the extent of adaptation to each component of the variable environment. By definition, the relative fitness of all lines was 1.0 at the beginning

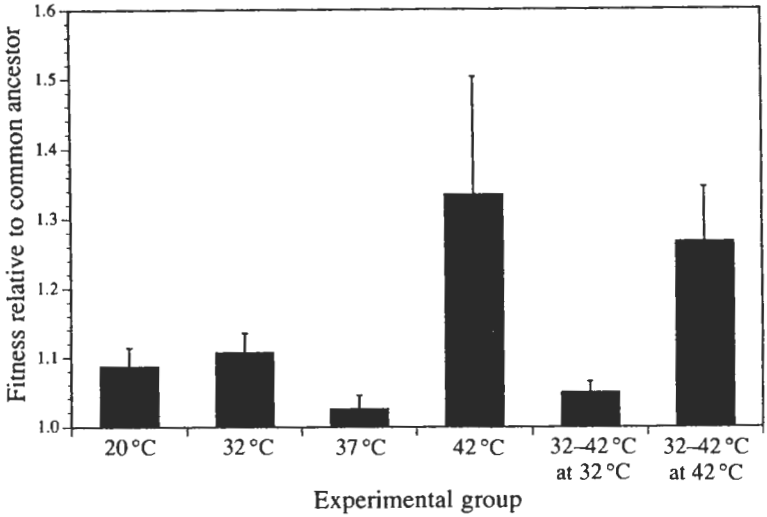

Fig. 2. Mean relative to their common ancestor for groups of *E. coli* populations that were propagated for 2000 generations at constant 20, 32, 37, or 42 °C or with daily alternation between 32 and 42 °C. Fitnesses were assayed at each group's selective temperature, or as indicated. The error bars represent 95% confidence intervals based on the six replicate lines in each group using a two-tailed *t*-distribution with $n-1 = 5$ degrees of freedom. (After Bennett & Lenski, 1996.)

of the experiment. The rate of adaptation was estimated by regressing relative fitness measured for each line over time (in generations). This rate of adaptation was significantly higher for all four of the groups experiencing novel thermal environments than for the control (37 °C) group, indicating that most of the adaptation that occurred was in response to the new thermal environments. The magnitude of the adaptive response was also highly variable between groups (Fig. 2). While there was no significant difference between the rates of adaptation to the environments below the ancestral temperature (i.e. 20 and 32 °C), the rate of adaptation to the high temperature environment (42 °C) was markedly greater. Similarly, in the 32–42 °C group, the rate of adaptation to the 42 °C component of the variable environment was substantially greater than that to the 32 °C component and was not significantly different from that of the 42 °C group to 42 °C. Therefore, the heterogeneity of the adaptive response to the different thermal regimens is not simply a function of the magnitude of the change from the ancestral temperature. An alternative explanation is that the

dependence of the mutation rate on temperature (Savva, 1982) is responsible for the increased adaptive response to the higher temperature environments. The data, however, do not fit this explanation either. First, there was no difference in the magnitude of the adaptive response between the 20 and 32 °C groups (Mongold *et al.*, 1996). Second, in the 32–42 °C group, variation generated by an elevated mutation rate during the days spent at the high temperature would be available for selection to act on it during the days spent at 32 °C. The rate of adaptation to the 32 °C component of the variable environment, however, was not elevated in that group (Lenski & Bennett, 1993; Lenski, 1995).

Correlated effects at other temperatures

It is generally recognised that temperature may be an important variable determining geographical distributions of species (Somero, 1995). What is less clear are the factors constraining species from evolutionary expansion of their ranges, as most natural populations have abundant genetic variation and hence apparent potential for adaptation to novel environments. One common view is that adaptation to novel environments is constrained by trade-offs in performance (Futuyma & Moreno, 1988). That is, genetic correlations among performance traits are assumed to be important across an environmental gradient, such that specialisation for performance in a novel environment may be associated with a decrement in performance in the ancestral environment or other environments. On the other hand, depending on the nature of the correlations, improvement in performance in a marginal environment might carry over to environments beyond the range of experience and therefore actually extend an organism's potential thermal niche. Although thermal trade-offs and other correlations between environments are widely assumed (Levins, 1968; Lynch & Gabriel, 1987; Pease *et al.*, 1989), there is little empirical support for their existence (for example, Huey & Hertz, 1984; but see Gilchrist, 1996). In our bacterial experiments, however, we were able to examine directly whether evolutionary (genetic) trade-offs occurred within the original ancestral thermal niche during adaptation. Furthermore, we could also examine whether a shift in thermal niche limits occurred during this thermal adaptation.

Improvement in relative fitness within the original thermal limits of the bacterium was very specific to the temperature in which the particular lines evolved. Among the groups maintained at a constant temperature (i.e. the 20, 32, 37 and 42 °C groups), the range of temperatures

over which each group improved relative to the ancestor usually extends only a few degrees in either direction from the temperature at which that group had evolved (Fig. 3). These groups may therefore be considered thermal specialists. The group maintained in a variable environment (32–42 °C group), on the other hand, showed significant improvement across a broad range of temperatures between its maximum and minimum daily temperatures, even extending to temperatures to which the lines had never been exposed (Fig. 3) (Bennett *et al.*, 1992). This group therefore comprises thermal generalists.

Although all of the specialist groups adapted to the greatest degree in their own thermal environment, the association of trade-offs in performance at other temperatures was highly asymmetrical with respect to the temperature of adaptation. In spite of the extensive adaptation of the groups which evolved at the higher temperature (42 °C), they suffered no significant loss in fitness at lower temperatures, even those below the ancestral temperature (Fig. 4). In contrast, adaptation to a much lower temperature (20 °C) was significantly correlated with a loss in relative fitness at higher temperatures. Similarly, the limits of the thermal niche, as measured by the temperature at which absolute fitness

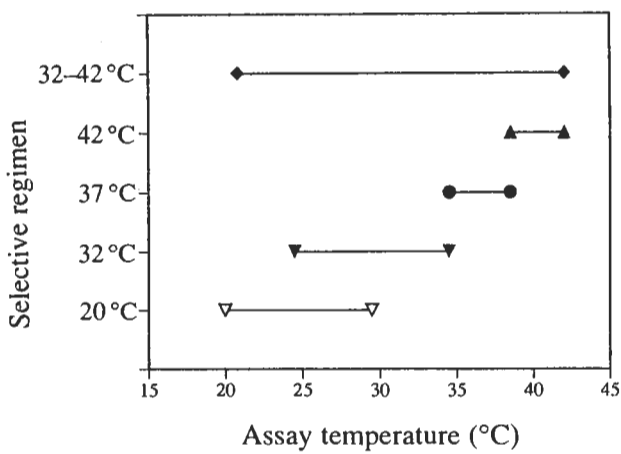

Fig. 3 Specificity of genetic adaptation with respect to environmental temperature in groups of *E. coli* populations that were propagated for 2000 generations at constant 20, 32, 37, or 42 °C or with daily alternation between 32 and 42 °C. Each solid line indicates the approximate range of temperature over which the mean fitness of a group was improved significantly relative to the common ancestor ($p < 0.05$). (After Bennett & Lenski, 1993; Mongold *et al.*, 1996)

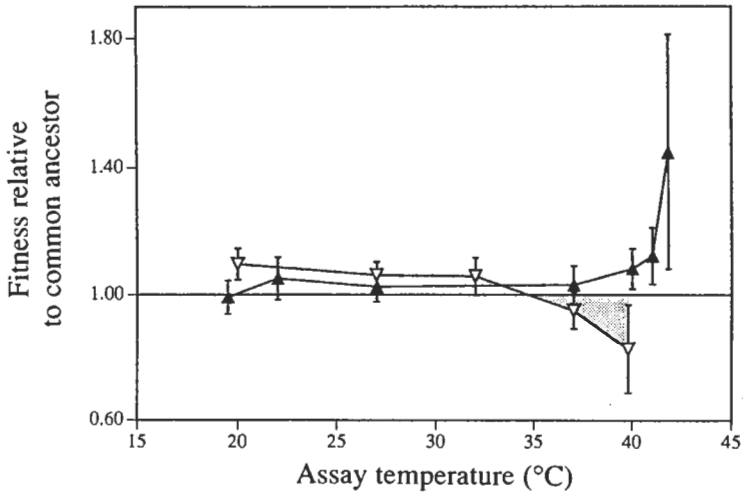

Fig. 4. Mean fitness of the 20 (▽) and 42 °C (▲) groups, relative to their common ancestor, measured across their thermal niche. The error bars represent 95% confidence intervals based on the six replicate lines in each group ($n-1 = 5$ d.f.). Note the trade-off in fitness of the 20 °C group when assayed at the higher temperatures (shaded area). (After Bennett & Lenski, 1993; Mongold *et al.*, 1996.)

became negative, were altered only in the low temperature adapted lines (Fig. 5): the lines of the 20 °C group experienced a downward shift in both their upper and lower thermal limits of 1–2 °C. None of the other groups had significantly altered thermal limits.

Evolutionary implications

Niche evolution

The potential thermal niche of an organism is shaped by long-term evolutionary forces acting in concert on the optimum temperature for performance, the breadth of the thermal range and the ultimate limits of thermal tolerance. An understanding of the correlations between these traits is fundamental to understanding the diversity of ecological types found in nature. For example, does evolutionary adaptation to a higher temperature result in a correlated shift in the entire range of thermal tolerance, a shift only in the optimum temperature for performance, or a broader niche with performance within the original thermal range being unaffected? Our experiments enable us to directly examine the effects of adaptation to altered temperatures on niche evolution (Fig. 5). Adap-

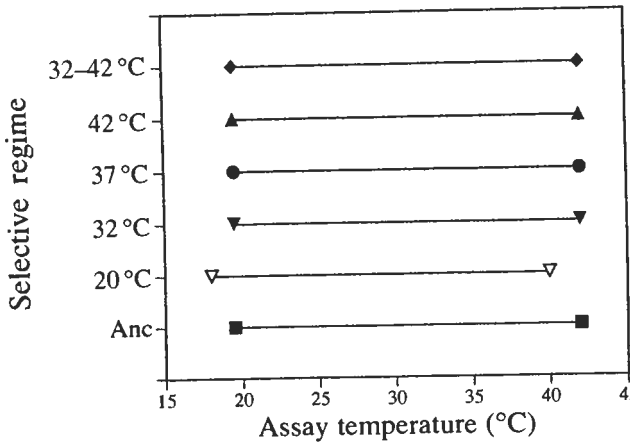

Fig. 5. Thermal niche of the ancestor and five experimental groups. Each solid line indicates the approximate range of temperatures over which the mean Malthusian parameter for a group was not negative, i.e. those temperatures where the lines were able to maintain a constant population density by replicating sufficiently to offset the daily serial dilution. The ancestral niche has been modified only in the 20 °C group. (After Bennett & Lenski, 1993; Mongold *et al.*, 1996.)

tation to 20 °C, very near the lower thermal niche boundary, resulted in both the lower and upper limits of thermal tolerance being shifted 1–2 °C lower. This group not only adapted to the selective temperature, but also became preadapted to even lower temperatures.

Performance at high temperatures was reduced in the process. In contrast, adaptation to a temperature very near the upper thermal limit, 42 °C, while greatly increasing fitness at that temperature (Fig. 2), did not appreciably shift either the upper or lower thermal limits from their ancestral condition. These results demonstrate the complexity of evolutionary changes in the niche. Starting with a single genotype of this single species, selection at or near the upper and lower niche limits produced completely different patterns of niche evolution. It is highly unlikely that any universal or predictable pattern of niche evolution will emerge for organisms in general if such contrasting effects can be observed within a single experimental system.

Origin and maintenance of diversity

The foregoing discussion examined how an organism's ecological potential might be affected by adaptation to a specific environment. Also of interest is the effect of adaptation on an organism's evolutionary

potential. Does selection in a homogeneous environment always result in parallel or convergent evolution of isolated populations? Or, will historical differences between populations send them down increasingly divergent evolutionary paths?

Historical differences may arise simply due to chance differences in the mutations which occur in separate populations. Lenski & Travisano (1994) maintained 12 replicate populations of bacteria for 10 000 generations at 37 °C in the same serial transfer environment described here. During the first 2000 generations, their populations similarly went through a period of rapid adaptation followed by a period of relative stasis. During the period of rapid change, the fitness of those populations relative to their common ancestor diverged significantly from one another and that divergence persisted for over 8000 generations. This suggests that, in spite of the fact that they experienced identical environmental conditions, the replicate populations acquired distinct adaptations with unequal effects on fitness.

Historical differences can also arise between populations which have adapted to different environmental conditions. In our study, we had groups of populations with 2000 generations of evolutionary history in different thermal environments. We could then ask whether historical contingencies had arisen which would constrain their future adaptive potential and promote continued divergence even if the populations subsequently experienced identical environments. To test this, the experimental lines previously adapted to 32, 37, 42 and 32–42 °C were moved to 20 °C and propagated in the same manner for an additional 2000 generations. After that period of time, all groups significantly increased their relative fitnesses at 20 °C, but there were no significant differences in the magnitude of this increase among the four groups with different thermal histories (Travisano *et al.*, 1995; Mongold *et al.*, 1996). Thus, in this case, specialisation for growth in different thermal environments had no directional impact on future potential for adaptation to this novel thermal environment. This conclusion must be qualified, however, as we do not know whether the outcome might have been different if the populations had been allowed to diverge for longer in their original selective environments.

Evolutionary response to stress

It has been proposed that physiological stresses, such as those caused by changes in climate, may accelerate the rate of adaptive evolution

(Parsons, 1987; Hoffman & Parsons, 1991). This proposition is based on observations of rapid divergence of local populations inhabiting marginal habitats on the edge of a species' geographical range or in semi-toxic environments (e.g. mine tailings with high concentrations of heavy metals) (Hoffmann & Parsons, 1991; Howarth, 1993). The proximate cause hypothesised for this rapid evolution is that physiological stress exposes a greater fraction of existing genetic variation to the action of selection.

A problem in testing this proposal is the difficulty in operationally defining and quantifying 'stress' in most biological systems (see Hoffmann & Parsons, 1991; Lenski & Bennett, 1993). Many of the criteria proposed for stress, both biochemical (e.g. stress protein formation) and ecological (e.g. growth rate and yield depression), can, however, easily be measured in bacteria. Our experimental system can therefore be used to illuminate the correlations between stress and evolutionary adaptation. Both 20 and 42 °C are very near the lower and upper thermal limits of population persistence of our ancestral bacterium. Environments near a niche edge are therefore expected to be stressful, and hence considered to be marginal. The intensity of selection might be expected to be equally intense for beneficial mutations occurring in both 'edge' environments. On the other hand, the biology of the bacteria at the two thermal boundaries is very different. The upper niche boundary is extremely sharp and characterised by a sudden shift from rapid growth to marked death between 42 and 44 °C. By contrast, the lower niche boundary is characterised by a gradual reduction in growth rate, which at about 19 °C becomes insufficient to offset the losses due to serial dilution. Lower temperatures are not lethal, but sufficiently inhibiting to slow growth rate. Growth rate is depressed at both 20 and 42 °C in comparison to 37 °C; in contrast, growth yield, biomass formed from available nutrients, is depressed only at 42 °C, not at 20 °C. Both temperatures induce a suite of 'stress response' genes in *E. coli* (Jones *et al.*, 1992; Craig *et al.*, 1993). At least 20 proteins are preferentially induced by shifts up in temperature (Neidhardt *et al.*, 1984; Delaney *et al.*, 1992). The functions of all of these proteins are not yet known, but they are believed to act, among other things, as molecular chaperones (Ellis & van der Vies, 1991; Martin *et al.*, 1991; Craig et al., 1993), aiding in the correct folding and oligomerisation of proteins. Following a downshift of 13 °C or more, the major cold shock protein, F10.6, is induced (Jones *et al.*, 1992). Induction of the cold shock response is believed to be negatively regulated by the level of (p)ppGpp and perhaps has some connections with the stringent response network (Jones *et al.*, 1992).

Therefore, by many criteria (e.g. niche edge, stress protein formation, growth rate depression), both 20 and 42 °C are stressful environments, although they differ with respect to induced mortality. The evolutionary response to those environments, however, was quite different. Adaptation was far more rapid and extensive in the high temperature environment, both in the 42 °C group and in the 32–42 °C group in the 42 °C environment (Fig. 2). In this environment, the expected match between stress and rapid evolution was indeed observed. The rate of adaptation to 20 °C, however, was considerably slower and no different from that at 32 °C, a very benign thermal environment that meets almost none of the criteria for stress. Stressful environments, therefore, do not invariably produce rapid rates of evolutionary adaptation.

Interestingly, adaptation to the lower temperature resulted in more far-reaching effects with regard to performance in other thermal environments. It entailed extensive trade-offs within the ancestral thermal niche and a downward shift of both upper and lower limits of the thermal niche, whereas adaptation to the high temperature was very temperature specific but produced neither trade-offs nor a shift in which limits (Figs. 4 and 5). Thus, rapid evolution associated with stress does not necessarily entail extensive changes in niche structure, but may be highly specific to a single environment.

The observations of an elevated rate of adaptation to high temperature, and the asymmetrical nature of the correlated responses associated with adaptation to high versus low temperature, may provide some insight into the targets of selection in these thermal environments. It appears that many of the traits that are important for performance at lower temperatures are functional across the thermal spectrum and are very temperature sensitive in terms of optimal performance. At high temperature, however, other functions may come into play that are not even expressed at lower temperatures. If these are the targets of selection, then their alteration may have relatively little impact on performance in other thermal environments.

Preadaptation

Adaptation entails the evolution of specific mechanisms that improve performance in a particular selective environment. These mechanisms may impact not only the functions selected but a host of correlated responses. These correlated responses may have only minor, or even no, functional consequences in the selective environment. If the population

subsequently colonises new environments or the original environment changes, however, then these formerly unimportant traits may differentially enable or disable the population in the new environment. Specifically, they may preadapt the population to the new circumstances, increasing fitness or even permitting persistence when it would otherwise be impossible. Alternatively, they may hinder performance in the new environment perhaps even dooming the population to eventual extinction if it cannot cope with the environmental change.

Although the concept of preadaptation is intuitively appealing, demonstrating its occurrence and generality, and investigating its properties can be problematic or impossible. Our bacterial system, however, demonstrates unequivocally that adaptation to one well-defined and characterised environmental factor, temperature, can entail widespread, genetically-determined divergence in functional capacities of other traits of no current selective value. Specifically, capacities to utilise the nutrient maltose, which was not present in the selective environment for at least 4000 generations and probably considerably longer, were greatly and differentially altered during temperature adaptation. Glucose was the only nutrient supplied during this evolutionary experiment, and glucose and maltose are transported across the outer and inner membranes by completely different pathways (Nikaido & Saier, 1992). Many of the adaptive mechanisms in our diverse thermal environments apparently involved changes in glucose transport, and these had a variety of correlated effects on the ability of the bacteria to utilise maltose as a nutrient. All possible responses were seen among the experimental lines (Fig. 6). In some, fitness in maltose was unaltered from the ancestral condition; in others, fitness declined; in still others, gains in fitness in maltose actually equalled or even exceeded those in glucose. In the 42 °C group, for instance, all individual lines had improved fitness in maltose, increasing the average fitness of the group in maltose by 55%. Some lines had as much as doubled fitness in maltose. Similar improvements in performance in maltose occurred in the 32–42 °C group at 42 °C.

For whatever mechanistic reasons, adaptation to a high temperature–glucose environment preadapted these experimental lineages to environments with maltose as the nutrient. If these lineages should now secondarily encounter maltose-containing environments, they will be more fit in these new environments than their ancestor as a result of temperature adaptation. Such preadaptation was not, however, an inevitable outcome of adaptation to novel temperatures: some individual lines of the 20 and 32 °C groups declined in fitness in maltose and were less able than their common ancestor to prosper competitively in

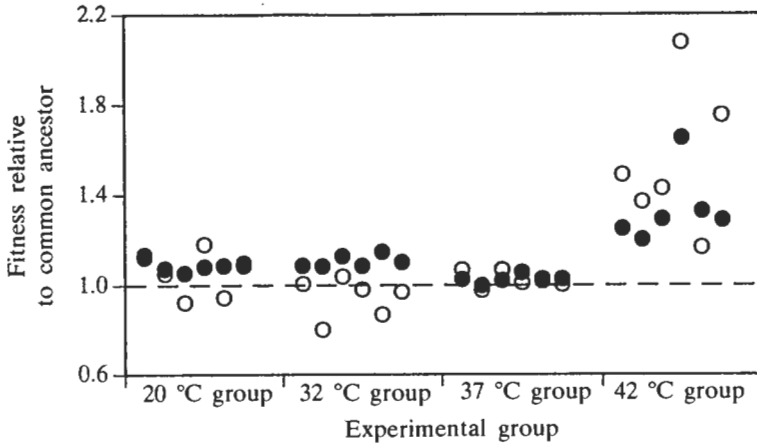

Fig. 6. Fitnesses of the individual lines in each of the four experimental groups selected at constant temperature (20, 32, 37 and 42 °C) relative to their common ancestor when grown in glucose (●) and maltose (○). (After Bennett & Lenski, 1993.)

those environments (Fig. 6). The differences in these correlated characters must depend on the exact mechanistic nature of the thermal adaptation in each lineage. They remain covert and unimportant, until the organisms find themselves tested in new environments.

Conclusion and perspectives

The study of evolutionary adaptation, as both process and product, is proving to be a fruitful field for interaction between organismal and evolutionary biology. The former provides a detailed understanding of the functional basis of adaptation, an appreciation for the integration of adaptive traits into all aspects of organismal function and a rich tradition of study of organism–environment interaction. Evolutionary biologists contribute a firm foundation in genetics and population biology and a rigorous theoretical and analytical approach to analyses of adaptation. Together, these formerly distinct fields can build a much richer and more comprehensive understanding of evolutionary adaptation than either could possibly accomplish separately. We have attempted to combine approaches and viewpoints from both traditions in our studies.

What specifically can be learned from our studies on evolutionary adaptation to temperature in bacteria? Our study system has strict

limitations: it was founded from a single strain of bacteria, indeed by a single genotype of that strain. Its populations were originally genetically homogeneous. Reproduction was strictly asexual, without the possibility of sexual recombination, so that all novelty had to arise *de novo* by mutation. This system cannot, therefore, serve as a model for the initial stages of adaptive evolution in genetically heterogeneous, sexually reproducing populations. In these, exposure to a new environment might favour a portion of the existing variability within the population, and adaptive novelty might arise through genetic recombination and be selected subsequently. Ultimately, however, the generation of novelty depends on mutation. Comparative studies on natural populations and species demonstrate that evolutionary adaptation to different thermal regimens depends on the origin of different alleles (examples reviewed by Hochachka & Somero, 1984; Powers, 1987; Somero, 1995). Our system permits the explicit study of the generation and consequences of such mutational novelty. This is not a general model for thermal adaptation in all biological systems: no single organism ever could be such, be it bacterium, plant, fly or mouse. The generality of the specific patterns obtained in our studies can be determined only by similar analyses on other systems.

The outstanding utility of this study system is in its ability to address and test a host of different general assertions about biological adaptation. *E. coli* is a typical mesophilic bacterium with a broad thermal niche and is not specialised for function in extreme thermal environments. It is therefore an excellent organism in which to examine the evolutionary responses to both moderate and stressful environmental change. Using this system, we have been able to conclude the following properties of evolutionary temperature adaptation:

1. Rates of adaptation (fitness improvement) can vary significantly among different novel thermal environments.
2. Adaptation can be highly temperature specific, often to a range of only a few degrees.
3. Adaptation and specialisation do not necessarily involve trade-offs in other environments.
4. Adaptation, even to niche extremes, does not necessarily involve a change in thermal niche.
5. Adaptation to stressful environments is not necessarily more rapid or extensive than adaptation to non-stressful environments.
6. Adaptation to an historical environment does not necessarily impede the rate of adaptation to a novel environment.

7. Correlated consequences of thermal adaptation may result in extensive preadaptation to other novel environments.

Several of these conclusions contradict widely held assertions (e.g. Levins, 1968; Hoffmann & Parsons, 1991) about patterns of evolutionary adaptation. If such evolutionary patterns are not found in this first detailed experimental test of their assertions, they are certainly not universal and are unlikely to be general. This is the particular utility of experimental evolution: it permits us to test propositions that we could formerly only assert.

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