AMER. ZOOL., 39:346–362 (1999)

Experimental Evolution and Its Role in Evolutionary Physiology[1]

ALBERT F. BENNETT[2] AND RICHARD E. LENSKI

Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92697
Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824

SYNOPSIS. **Four general approaches to the study of evolutionary physiology—phylogenetically-based comparisons, genetic analyses and manipulations, phenotypic plasticity and manipulation, and selection studies—are outlined and discussed. We provide an example of the latter, the application of laboratory selection experiments to the study of a general issue in environmental adaptation, differences in adaptive patterns of generalists and specialists. A clone of the bacterium *Escherichia coli* that had evolved in a constant environment of 37°C was replicated into 6 populations and allowed to reproduce for 2,000 generations in a variable thermal environment alternating between 32 and 42°C. As predicted by theory, fitness and efficiency of resource use increased in this new environment, as did stress resistance. Contrary to predictions, however, fitness and efficiency in the constant ancestral environment of 37°C did not decrease, nor did thermal niche breadth or phenotypic plasticity increase. Selection experiments can thus provide a valuable approach to testing hypotheses and assumptions about the evolution of functional characters.**

> "Karl Marx seems today a shadowed and limited historical figure, whereas Darwin is entirely contemporary. What he learned may affect everything we desire and everything we forget, what we wish to do and what we do not wish to do."
>
> David Denby, 1997

The organismal sciences, including physiology, morphology, and behavior, have all undertaken an analytical transition in recent years. Lauder (1981) has characterized this progression as leading from equilibrium into transformational studies. The former examine organismal structure and function in interaction with the current environment; the latter incorporate an historical, explicitly evolutionary, perspective in their analyses. Darwin's ideas continue today to have a major impact on these areas of organismal biology, affecting what we desire to learn and what we wish to do and do not wish to do. The inclusion of this evolutionary component is a welcome addition for organismal biology, because it broadens our questions and the richness of our understanding of the complexity of and constraints on structure and function (Bennett, 1997). It is also a positive development for the field of evolutionary biology itself (Futuyma, 1998), because the characters that organismal biology studies, such as energy use, locomotor and feeding performance, and reproductive behaviors, are the very factors that are expected to be of primary importance for fitness. These major areas of biology which formerly had little interaction have begun a mutually informative dialogue.

APPROACHES TO STUDYING EVOLUTIONARY PHYSIOLOGY

In the area of comparative physiology and physiological ecology, this evolutionary emphasis has been recognized by the application of a new name to the field, evolutionary physiology (Diamond, 1992, 1993; Garland and Carter, 1994). It is important to recognize, however, that interest in and investigations of the evolution of physiological characters antedate the designation "evolutionary physiology" (*e.g.*, Watt, 1977; Snyder, 1981; DiMichele and Powers, 1982).

---

[1] From the Symposium *Evolutionary Physiology* presented at the Annual Meeting of the Society for Integrative and Comparative Biology, 3–7 January 1998, at Boston, Massachusetts.

[2] E-mail: abennett@uci.edu

Most frequently, major changes in a field such as the physiological sciences are technologically driven. That is, the invention or application of a new technique or apparatus permits more accurate measurements or determination of new variables, leading to new insights and questions. But evolutionary physiology is not technologically driven in this manner. What is novel about this approach is not the type of characters examined or the methods used for their investigation. The variables examined, such as metabolic rate, locomotor speed, thermoregulatory performance and the physiological mechanisms that underlie them, are those that comparative physiology and physiological ecology have measured for decades. What is new is the analytical context for these studies. Familiar characters are interpreted within the framework of new questions and analytical paradigms, leading to new approaches to the study of organismal characters. This interest in the evolution of physiological traits was given major impetus by the publications of Gould and Lewontin (1979), Felsenstein (1985), and Feder *et al.* (1987).

The major ways in which evolutionary physiology has developed and is studied are briefly detailed here. Macroevolutionary studies, those that explore adaptations and character evolution among species or higher level taxa, are usually (by necessity) approached through historically-based comparisons. Microevolutionary studies, those examining the dynamics of selection and evolutionary change within populations, may be undertaken through genetic, phenotypic, or selection approaches.

Historically-based comparisons

Currently, the most familiar and widely-used paradigm in evolutionary physiology is the interpretation of physiological characters within an historical, comparative framework. In these studies, physiological and/or ecological observations are analyzed along with an independently-derived phylogeny of the taxa involved. Such factors as ancestral state, rates of evolutionary change, and adaptive implication are then analyzed according to patterns of character distribution across taxa (see Felsenstein, 1985; Harvey and Pagel, 1991; Garland, 1999 for a discussion of methods and interpretations). Taxa involved in these analyses may be populations, species, genera, or even higher levels of biological organization, or may encompass a combination of different organizational levels. Because of the necessity of incorporating a more distantly related "out group" into any comparison, a minimum of three different taxa is required in any such analysis (Garland and Adolph, 1994). Phylogenetic methods have been successfully applied to study the evolution of a wide variety of physiological characters, including thermoregulatory pattern in fish (Block *et al.*, 1993), locomotor performance in lizards (Losos, 1990), diving physiology of pinnipeds (Hochachka, 1997), metabolic rate in amphibians (Walton, 1993), expression of glycolytic enzymes in fish (Pierce and Crawford, 1997), plasma osmotic concentration in amniotes (Garland *et al.*, 1997), and anaerobic metabolic endproducts in chordates (Ruben and Bennett, 1980), to mention only a few of dozens of such studies.

In addition, most studies of paleobiology that discuss the evolution of functional characters also implicitly use this approach, and can be considered to employ historically-based comparisons. Even though they may not include an explicit phylogenetic analysis, their arguments are based on shared characters, parsimony, and assumed ancestral condition (Bennett and Ruben, 1986) and in the end amount to the same thing.

Comparatively-based phylogenetic studies have the great advantages of being able to examine very diverse taxa that have evolved in natural environments with all their complexity. However, they also have the interpretive difficulty of all comparative studies—the analysis of correlation, not causation. In addition, their interpretation depends crucially on the assumption of the underlying phylogenetic relationships, which themselves depend on assumptions of parsimony. Although phylogenetically-based studies are a major improvement in the analysis of comparative data, it should be borne in mind that their phylogenetic bases are additional sources of uncertainty for data interpretation.

Genetic analyses and manipulations

For selection to occur and for evolutionary change to proceed, there must be heritable phenotypic variation within a population. Investigations of the genetic basis of physiological traits has thus assumed a major importance in evolutionary physiology. Two types of approach have been used in these studies: the investigation of the genetic basis of functional traits and the influence of single genes on organismal characters. Arnold (1987) has characterized these two perspectives as physiology-to-genetics and gene-to-physiology, respectively, depending on their primary analytical context. In the former mode, evolutionary physiologists applied the techniques and insights of quantitative genetics (see Falconer, 1996; Lynch and Walsh, 1998) to estimate the heritability of a variety of functional characters, both organismal and suborgansimal. Particular attention has been directed to studies of locomotor performance (*e.g.*, Tsuji *et al.*, 1989; Sorci *et al.*, 1995; Dohm *et al.*, 1996) and its mechanistic basis (*e.g.*, Garland and Bennett, 1990; Garland *et al.*, 1990). Gene-to-physiology studies examine the performance and fitness implications of single genes on organismal characters, for example, lactate dehydrogenase on locomotor performance (Powers, 1987; DiMichele *et al.*, 1991) and temperature adaptation (Somero, 1995; Holland *et al.*, 1997; Fields and Somero, 1997), hemoglobin on oxygen transport capacity (Snyder, 1981), aminopeptidase on osmoregulatory ability (Koehn *et al.*, 1980, 1983), and phosphoglucose isomerase (Watt, 1977; Watt *et al.*, 1983, 1996) and glycerol-3-phosphate dehydrogenase (Barnes and Laurie-Ahlberg, 1986) on flight capacity. Other workers have been concerned with investigating more global genetic issues concerning organismal performance, such as pleiotropic effects on the evolution of physiological characters (Service *et al.*, 1985; Graves *et al.*, 1992; Djawdan *et al.*, 1996), the role of overall heterozygosity in influencing performance and fitness (Koehn, 1987; Mitton, 1994), the effects of genetic correlation on rates of evolutionary change (Arnold, 1987), and the relationship between numbers of deleterious mutations and overall viability and fitness (Mukai, 1969; Elena and Lenski, 1997).

Of particular promise for the future of these genetic studies is the possibility of manipulating specific genes using the techniques of modern molecular biology. If this approach is employed to understand the evolution of a character (as opposed to a mechanistic investigation of gene function, as is now common in molecular biology), it can provide a particularly powerful tool in evolutionary physiology. Such manipulation would permit the experimental testing of hypotheses generated in comparative studies, such as the role of a specific enzyme influencing a cascade of physiological and organismal characters (*e.g.*, the gene-to-physiology studies cited above). Most such studies to date have been performed with bacteria owing to their ease of genetic manipulation. A few examples are the consequences of excess tryptophan synthesis for bacterial growth (Dykhuizen, 1978), the effects of lactose-permease and β-galactosidase expression on metabolic flux in bacteria (Dykhuizen and Dean, 1990), and interactions between structural and regulatory genes that control expression of an efflux protein and their effects on bacterial growth in the presence and absence of an antibiotic (Nguyen *et al.*, 1989; Lenski *et al.*, 1994). An example of similar studies in eukaryotes is the experimental test of the role of stress proteins in conferring heat tolerance in fruitflies (Feder *et al.*, 1996). Similar genetic manipulations will undoubtedly be one of the most exciting and productive areas in future studies of evolutionary physiology.

Phenotypic plasticity and manipulation

Somewhat unexpectedly, evolutionary physiological studies have renewed interest in the range of phenotypic responses to different environments, such as thermal acclimation, which have been a staple of comparative physiological studies for a very long time. In this context, interest is centered on phenotypic plasticity as an alternative to genetic change during evolution.

If an organism can effectively cope with environmental change through phenotypic responses, evolutionary change is unnecessary, and phenotypic plasticity becomes an alternative to evolution. This view presumes that phenotypic alterations in different environments are inherently beneficial in those environments (Cossins and Bowler, 1987; Hoffmann and Parsons, 1991). Particular interest has recently been directed to testing this assumption experimentally (*e.g.*, Leroi *et al.*, 1994*a*; Huey and Berrigan, 1996; Huey, 1999). These studies attempt not only to document phenotypic plasticity, but also to determine its fitness implication and whether it is indeed an effective alternative to evolution.

Another approach to studying the importance of phenotypic characters is their direct manipulation and subsequent study of their performance and/or fitness consequences (Sinervo and Basolo, 1996). These studies encompass the alteration of particular characters of organisms, such as wing coloration (*e.g.*, Kingsolver, 1995, 1996), intestinal transport capacity (Buchmiller *et al.*, 1993; Hammond *et al.*, 1996*b*), milk production capacity (Hammond *et al.*, 1996*a*), or manipulation of total body size (Sinervo, 1990; Sinervo *et al.*, 1992). The latter approach has been designated allometric engineering (Sinervo and Huey, 1990). Performance consequences of such changes can be examined either in staged encounters in the laboratory or in animals released into natural populations in nature. These latter studies then become a type of natural selection study, as discussed in the next section. Phenotypic manipulation studies permit a direct experimental test of the significance of a character and are thus valuable tools to expand upon comparative studies.

Selection studies

Differential survival and reproductive success is the driving force of evolutionary change. Direct observation and/or manipulation of characters under selection is thus a particularly powerful tool for studying evolutionary physiology. Selection studies permit one to watch evolution in action, not just to speculate on its operation through its consequences. Further, selection studies permit direct experiments to test *a priori* hypotheses about evolution (Bell, 1997*a*, *b*). Because these approaches are so powerful and have not previously been widely used within the fields of comparative physiology and physiological ecology, the remainder of this article will concentrate on selection studies and provide an example of their utility in testing general evolutionary hypotheses.

Selection studies may be divided into two broad categories: *natural selection in the wild* and *selection in the laboratory.* The former approach has been extensively reviewed by Endler (1986). Differential survival or reproductive success among individuals is measured in a natural population of organisms and is correlated with possession of a particular character to determine whether it may be under natural selection. Classic studies of natural selection in the wild include those on wing and body size in sparrows (Bumpus, 1899), beak morphology in Galapagos finches (Grant *et al.*, 1976; Grant and Grant, 1993), and color pattern (Endler, 1980, 1983) and life history characters (Reznick and Endler, 1982; Reznick *et al.*, 1990) in guppies. In Endler's (1986) review, he stated (p. 159) "There has been very little interest by physiologists in variation within species or in the significance of that variation; therefore, natural selection on physiological variation is largely unexplored." Although evolutionary physiologists have subsequently become very interested in variation in physiological characters within populations (Bennett, 1987), still relatively few studies on selection on physiological characters in natural populations have been undertaken (*e.g.*, a few studies on the selective importance of locomotor performance capacity: Miles, 1987; Jayne and Bennett, 1990; unpub. data of Huey *et al.* in Bennett and Huey, 1990). Endler's observation remains correct: the area is still largely unexplored.

The other group of selection studies involves the observation of evolution under controlled conditions in the laboratory. Three different designs of laboratory selection experiments have been recognized (Rose *et al.*, 1990). The first is *laboratory natural selection,* in which replicated populations are exposed to some alteration in an environmental variable (such as temperature or osmotic concentration) and changes within the populations over many generations are measured and analyzed. The experimenter provides the environment, but does not otherwise directly select on any character or choose individuals for differential breeding: the populations are left to their own devices to evolve solutions to the environmental challenge. Examples of this type of study are thermal evolution experiments on protists (Dallinger, 1887), bacteria (Bennett *et al.*, 1992; Mongold *et al.*, 1996*a*), and fruit flies (Huey *et al.*, 1991; Partridge *et al.*, 1995). The second variety is *artificial truncation selection,* in which only organisms possessing certain characters (or character values of an extreme nature) chosen a priori are permitted to reproduce and found the next generation. This artificial selection is the familiar form used in animal and plant breeding, and it can be very expeditious in quickly producing certain desirable phenotypes. However, by imposing a single performance criterion for success, it may constrain the pathways along which evolution could proceed to solve the more general problem. An example of this experimental design in an evolutionary physiological context is selection on voluntary running behavior in mice (Swallow *et al.*, 1998*a*, *b*). The third approach is *laboratory culling*. In this design, an extreme environmental condition (*e.g.*, high temperature or desiccation) is imposed every generation, and only a small proportion of the population is permitted to survive to reproduce the next generation. This type of selection has elements of the other two designs. It typically permits only a small fraction of the population to breed by imposing a selective screen, in contrast to most laboratory natural selection protocols. However, it does not specify that survivors must possess certain phenotypic characters, as does artificial truncation selection; consequently, a diversity of pathways might be used in evolving solutions to the environmental challenge. This type of selection has been used to study the evolution of heat tolerance (Huey *et al.*, 1992; McColl *et al.*, 1996; Loeschcke and Krebs, 1996), desiccation resistance (Hoffmann and Parsons, 1989; Gibbs *et al.*, 1997), and longevity (Rose, 1984; Chippendale *et al.*, 1994) in fruit flies.

If all things were equal, in evolutionary physiology one would obviously want to undertake selection studies on natural populations in natural environments. These would then reflect the complexity and richness of the real world, and would accord well with the strong ethic of physiological ecology concerning the importance of field studies. But all things are far from equal. Such studies are very time and personnel intensive, as physiological characters are often difficult to measure expeditiously on large numbers of organisms under field conditions. These studies are also usually handicapped by lack of replication and control, limiting the conclusions that can be drawn from them. Disentangling genetic and environmental influences on phenotypes in the field is notoriously difficult. And the number of generations over which selection can be observed in most natural populations is extremely limited. While studies of selection on physiological characters in natural populations have obvious appeal, their practical difficulties will probably always constrain their frequent undertaking and limit the quality and richness of the data that can be obtained.

In contrast, although the laboratory environment lacks the complexity of the natural world, it offers important compensations, including the ability to control experimental conditions and to replicate experiments, permitting the rigorous evaluation of a priori hypotheses. Large population sizes can be maintained, minimizing the effects of drift on experimental outcome. In some types of organisms (*e.g.*, viruses, bacteria), evolution can be studied over hundreds and even thousands of generations; moreover, the ancestral organisms can be preserved in a state of suspended animation and later revived to permit measurements of fitness through direct competition with their own descendants. Although only certain groups are amenable to selection experiments (those that can be mass-cultured in the laboratory and have short generation times), they span a very large taxonomic range, including viruses, bacteria, fungi, protists, nematodes, arthropods, and even mice, so that patterns of evolutionary change to similar environments can be investigated in diverse groups of organisms. Studies on selection in the laboratory can thus valuably complement studies of natural selection in the wild, extend studies of selection in terms of both duration and strength of inference, and provide direct tests of assumptions and hypotheses concerning environmental adaptation.

Laboratory selection studies can be particularly powerful tools for evolutionary physiology because of their great versatility. They can be used as a means of generating new biological material for study, that is, literally building a better mouse in order to analyze its performance and underlying physiology. The studies of Garland and his coworkers are exemplary in this regard (Swallow *et al.*, 1998*a*, *b*): selection on voluntary running performance in replicated lineages of mice has resulted in substantial increments in maximum oxygen consumption in comparison to sedentary controls. It is now possible to examine exactly which portions of the oxygen transporting and utilization systems have responded to selection and the mechanisms responsible for the increased oxygen consumption ability. The replicated lineages make it possible to ask whether similar physiological changes occur repeatedly or whether a variety of solutions are employed in response to this selection. The availability of control lineages provides an unambiguous assessment of the original, pre-selective condition, permitting easier isolation and analysis of the physiological changes that have occurred.

As valuable as this ability to produce and analyze new genetic variation is, in our opinion the principal utility of studies of selection in the laboratory is their ability to permit experimental tests of general assertions or hypotheses about patterns and consequences of evolutionary adaptation. The mouse experiments, in addition to the analysis of physiological mechanisms that they permit, can be used to test a range of hypotheses about physiological evolution, including those concerning symmorphosis, the aerobic capacity model for the evolution of endothermy, and performance tradeoffs. To be specific, symmorphosis (Taylor and Weibel, 1981; Weibel *et al.*, 1991) might predict coordinated and simultaneous evolution in all parts of the oxygen transport and utilization systems, as opposed to alteration of only one or two rate-limiting factors. The aerobic capacity model (Bennett and Ruben, 1979) predicts an evolutionary linkage between maximal and minimal rates of oxygen consumption; this hypothesis predicts a coordinated increase in basal metabolic rates in the activity-selected mice. A tradeoff between the locomotor performance modes of burst activity and endurance are commonly assumed, and a negative genetic correlation between them has been demonstrated in mice (Dohm *et al.*, 1996). This assumption predicts that burst speed should be decreased in the groups of mice selected for endurance activity. In each of these instances, the utility of this kind of selection experiment in examining larger issues in evolutionary physiology is clear.

TESTING EVOLUTIONARY HYPOTHESES IN THE LABORATORY: GENERALISTS *VS.* SPECIALISTS

To illustrate the application of laboratory selection studies to general issues in evolutionary physiology, we will use data from our ongoing experiments on adaptation of bacteria to different thermal environments. Specifically, we will investigate adaptation to constant and variable environments, a potential mechanism for differentiation between evolutionary specialists and generalists. The consequences of evolution in variable (or changing) environments have been the subject of considerable theoretical speculation among evolutionists (*e.g.*, Levins, 1968; Sibly and Calow, 1986; Hoffmann and Parsons, 1991; Gilchrist, 1995). Most of these studies postulate inevitable tradeoffs between specialization and niche breadth (Futuyma and Moreno, 1988), such that specialist organisms from constant environments are predicted to have narrow niches and lower functional capacities outside their own selective environments (*e.g.*, eq. 2.1 in Levins, 1968; eq. 3 in Gilchrist, 1995). Conversely, generalists, because they must cope with many different environments, are predicted to be less successful in functioning in any particular one. These viewpoints may be summarized facilely as "use it or lose it" for the specialist and "a jack-of-all-trades is a master of none" (Huey and Hertz, 1984) for the generalist.

These mirror-image perspectives form a coherent pair, and they make numerous concrete predictions about evolution in constant and varying thermal environments. In particular, if we consider a population of thermal specialists that is subsequently exposed to and evolves in a varying thermal environment, the following changes are anticipated: (i) *Fitness in the variable environment will increase.* In particular, reproductive success is predicted to increase in the portions of the new environment not previously experienced. Stated very simply, the population is predicted to adapt to its new environment. (ii) *Efficiency of resource use in the variable environment will increase.* One potential way in which fitness could increase in the new environment is through an improvement in the ability to convert energy resources into biomass. While such an improvement in efficiency is not an absolutely necessary correlate of adaptation, it is a reasonable pathway by which it might occur. While some authors (*e.g.*, Heinrich, 1996) argue that adaptation inevitably results in improved efficiency, others have pointed out that natural selection could favor a decrease in efficiency of resource use, if it were functionally coupled to an increased reproductive rate (Harold, 1986). (iii) *Fitness in the ancestral specialized environment will decrease.* Because a jack-of-all-trades must cope with many new and different environments, it should no longer be as good as it was in its original environment, even if that ancestral environment is a component of its new variable environment. (iv) *Efficiency of resource use in the ancestral specialized environment will decrease.* Again, this is not a necessary correlate, but is a potential pathway to the decreased fitness anticipated in the previous prediction. (v) *Thermal niche breadth will increase.* As the range of environments actually experienced broadens, the range of environments in which the adapted population can persist is predicted to expand even further. (vi) *Resistance to stress will increase.* Variable environments may encompass stressful conditions. Thus, the ability to cope with increased stress may also increase as a consequence of adaptation to variability. In the example provided, the variable selective regime does include such a stressful component. (vii) *Phenotypic plasticity will increase.* Because change is inherent in a variable environment, it is predicted that the evolving organisms will have enhanced ability to change their phenotypes in ways that will improve their performance in the face of environmental change. We will test this entire suite of predictions by examining several lineages of bacteria that have adapted to different thermal environments in laboratory selection experiments.

Experimental organisms

We used lineages of the bacterium *Escherichia coli* that were cultured serially in a dilute medium in which glucose was the only source of carbon and energy. Each day the population was diluted 100-fold into fresh nutrient medium. Sufficient glucose was provided to permit a 100-fold increase in population size each day, such that the population underwent an average of about 6.6 generations of binary fission per day ($\log_2 100 \approx 6.6$). The design of these experiments is reported in Bennett *et al.* (1992) and Mongold *et al.* (1996*a*), and the reader is referred to those studies for more detailed information on methods of culture, exclusion of contamination, measurement of fitness, etc. The results of several subsequent studies examining the properties of these evolutionary lineages are reviewed in Mongold *et al.* (1996*b*).

Our experiments investigating thermal adaptation began with a bacterial lineage that had been exposed to a constant temperature of 37°C for 2,000 generations (Lenski *et al.*, 1991). During this time, its fitness, measured as its net reproductive rate, increased by about 30%. During a subsequent 2,000 generations at this same temperature, its fitness further increased by only about 2% (Bennett and Lenski, 1996). Thus, it had attained substantial adaptation to this environment and can be considered a true thermal specialist in a constant thermal environment. We cloned a single bacterium from this lineage, which we designate as the *ancestor*. This ancestor was used to found five different experimental *groups*, each consisting of six replicate *lines*, which were maintained in different thermal environments for another 2,000 generations. These groups consisted of lines in four constant environments, 20, 32, 37, and 41–42°C (here designated 42°C for brevity), and one variable environment, 32–42°C, which consisted of daily alternating exposure to 32°C and then to 41–42°C. It is this latter group that is of particular interest in regard to questions of evolution in a variable thermal environment and its attendant consequences. Here we will examine the patterns of adaptation of the 32–42°C group in comparison to those of its own ancestor and use these to test the previously described predictions concerning the evolution of generalists and specialists.

Testing hypotheses: Fitness in the variable environment will increase

A population that had experienced nothing but a constant thermal environment was suddenly exposed to daily temperature changes of 10°C and propagated with such changes for 2,000 generations. Did adaptation to this new environment occur? That is, did fitness, measured as reproductive rate, increase in this new thermal regime? A distinct experimental advantage of bacteria is their ability to survive freezing, so that the ancestor of an experimental lineage can be stored indefinitely and then later revived to compete directly with its descendant. Thus, competition for the limiting resources and attendant reproductive output permits a direct measurement of fitness of the descendant organism relative to its own ancestor. Fitness is calculated simply as the ratio of the number of cell divisions of a descendant organism relative to that of its ancestor: a value of 1 indicates no change in fitness, whereas a value significantly above 1 demonstrates adaptation. These measurements of fitness can be done in any environment, including the ancestral or the new selective environment, to permit a quantitative assessment of adaptation and its consequences.

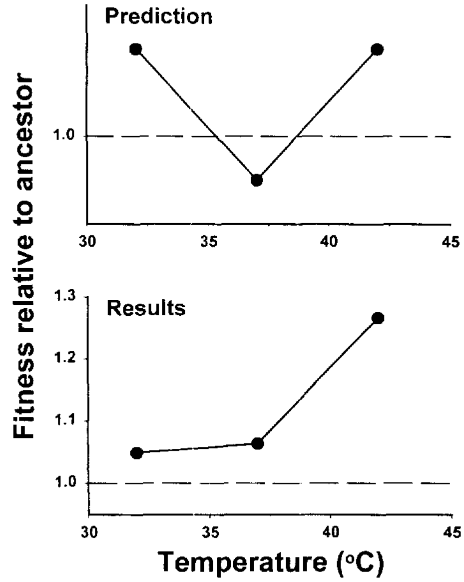

FIG. 1. Predicted and actual changes in fitness during the evolution of the thermal generalist group. Mean fitness values for 6 lines are reported and are significantly greater than ancestral fitness at all three temperatures. The predicted improvements at 32°C and at 42°C occurred, but the tradeoff in fitness at 37°C did not occur. Data from Bennett et al. (1992) and Bennett and Lenski (1996).

When the ancestor and each of the six replicate lines of the 32–42°C group compete in the new variable thermal environment of one day at 32°C and then one day at 42°C, the fitness of each of the derived lines is greater than that of the ancestor (Bennett and Lenski, 1996). The mean relative fitness (W) of all six lines is 1.174 (± 0.068 95% C.L., $P = 0.001$), supporting the original (if unsurprising) hypothesis that fitness in the new variable thermal environment did indeed increase. *Hypothesis supported.* It is interesting, however, that the fitness increase was not symmetrical in the two components of the new environment (Bennett and Lenski, 1996) (Fig. 1). Adaptation was considerably greater to the warmer portion of the environment (W at 42°C $= 1.267 \pm 0.079$ 95% C.L.) than to the cooler portion (W at 32°C $= 1.049 \pm 0.016$, 95% C.L.), and this difference itself is highly significant ($P = 0.001$, paired t-

TABLE 1. *The efficiency of resource use by the ancestor and the average of six lines of the 32–42°C group, measured at 32°C and at 42°C.**

Temperature °C	mJ bacteria/mJ glucose		
	Ancestor	32–42°C Group	*P*
32	0.401 ± 0.005	0.469 ± 0.018	<0.0001
42	0.176 ± 0.008	0.315 ± 0.041	<0.0001

*Mean efficiencies and 95% confidence limits are reported; n = 20 replicate assays for ancestor, n = 6 independent lines of the 32–42°C group; *P* based on *t*-test. Unpublished data from Bennett and Lenski.

test). Thus while the presumption of adaptation was fulfilled, the exact pattern that the adaptation may take is less easy to predict.

Testing hypotheses: Efficiency of resource use in the variable environment will increase

Did the observed fitness improvement entail an increase in the conversion efficiency of nutrient resource to biomass? Each day the population is provided a constant amount of glucose, and thus the amount of energy available is known. The energy content of the resulting population growth can be calculated from measurements of the number and size of cells, given standard assumptions about their energy content (Bennett and Lenski, unpub. data). Thus, the efficiency of resource use and its evolution can be determined.

The efficiency of resource use of the ancestor and of the 32–42°C group in the two component temperatures of the variable thermal environment are reported in Table 1 and Figure 2. The amount of energy converted to biomass per unit of nutrient energy increased significantly at both temperatures, indicating that fitness improvement in the variable thermal environment was at least partially due to increased efficiency. *Hypothesis supported.* Note also that conversion efficiency is very temperature dependent over this range (Table 1), with markedly decreased efficiencies at higher temperatures, as has been previously reported for other species (*e.g.*, Senez, 1962). The evolutionary improvement in efficiency was more marked at the higher temperature, with the amount of biomass formed from a constant amount of nutrient increasing 79%, compared with a 7% increase at the lower temperature.

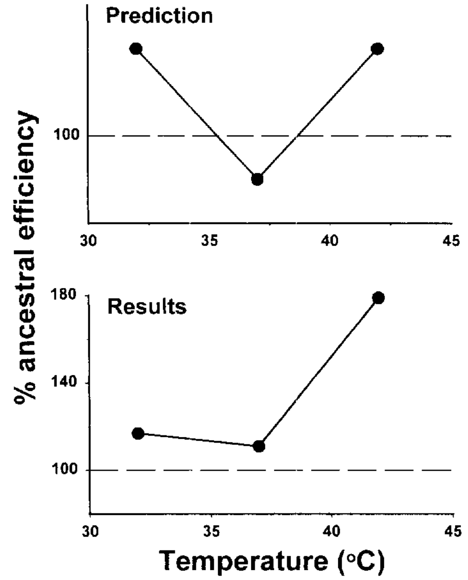

FIG. 2. Predicted and actual changes in efficiency of resource use during evolution of the thermal generalist group. Efficiencies are reported as percentage of ancestral values [(mJ bacteria formed/mJ glucose) × 100]. Mean values for 6 lines are given and are significantly greater than ancestral efficiencies at all three temperatures. The predicted gains at 32°C and at 42°C occurred, but the tradeoff in efficiency at 37°C did not occur. Unpublished data of Bennett and Lenski.

Testing hypotheses: Fitness in the ancestral specialized environment will decrease

The assumption of tradeoff predicts that as fitness increases in the generalist variable environment, it will decrease in the specialized ancestral environment. Thus, mean fitness of the evolving lines of the 32–42°C group at the ancestral temperature of 37°C is predicted to decrease (*i.e.*, W is expected to decline significantly below 1). However, mean W of the six lines of the group at 37°C is 1.064 (±0.036 95% C.L.), indicating a significant *increase* ($P = 0.006$ based on *t*-test), rather than a decrease, in mean fitness at the ancestral temperature (Bennett *et al.*, 1992) (Fig. 1). *Hypothesis not supported.* Evidently a fitness tradeoff in the ancestral specialized environment is not a necessary prerequisite for adaptation to a broader thermal environment.

Testing hypotheses: Efficiency of resource use in the ancestral specialized environment will decrease

Because mean fitness in the ancestral environment did not in fact decrease, this prediction loses its underlying assumption. It is therefore not particularly surprising that efficiency of resource use at 37°C did not decline as predicted, but rather it increased (Bennett and Lenski, unpub. data) (Fig. 2): mJ bacteria formed/mJ glucose at 37°C is 0.402 (±0.008 95% C.L., $n = 20$ assays) in the ancestor and increased to 0.446 (±0.022 95% C.L., $n = 6$ lines) in the 32–42°C group ($P < 0.0001$ based on t-test). *Hypothesis not supported.*

Testing hypotheses: Thermal niche breadth will increase

It is anticipated that thermal generalists should have broader thermal niches than thermal specialists (*e.g.*, Prosser, 1991). It was therefore predicted that thermal niche breadth should increase during adaptation to the 32–42°C environment. Thermal niche breadth in our experimental system is defined as the range of temperatures over which the population can maintain itself in serial dilution culture (Bennett and Lenski, 1993). This maintenance requires 100-fold reproductive increase each day, which offsets the 100-fold dilution that is part of the selective regime. If the population grows, but not 100-fold, then it will be diluted from culture and go extinct. The thermal niche of the ancestor, which is adapted to constant 37°C, extends from 19.5°C to about 42°C (Bennett and Lenski, 1993). Thus, the thermal generalist lines, which were maintained at 41–42°C for half of their 2,000 preceding generations, experienced a temperature within 1°C of their ancestral upper limit. Consequently, an extension of that limit in particular might have been anticipated. However, the thermal niche of the 32–42°C group was essentially unaltered, remaining from 19.5°C to 42°C (Bennett and Lenski, 1993) (Fig. 3). *Hypothesis not supported.* Apparently, niche extension is not an inevitable result of an adaptive change from a constant to a variable thermal environment. It is noteworthy in this regard that these bacteria are, in fact, capable of thermal niche evolution. The group of lines evolving at 20°C experienced a significant decrease in both their lower and upper thermal limits (Mongold *et al.*, 1996*a*), and some mutants are able to grow at 43°C (Bennett and Lenski, 1993; Mongold, Bennett, and Lenski, unpub. data). Hence, the failure of the generalist lines in the 32–42°C group to expand their thermal niche is not because of some absolute constraint on growth at extreme temperatures.

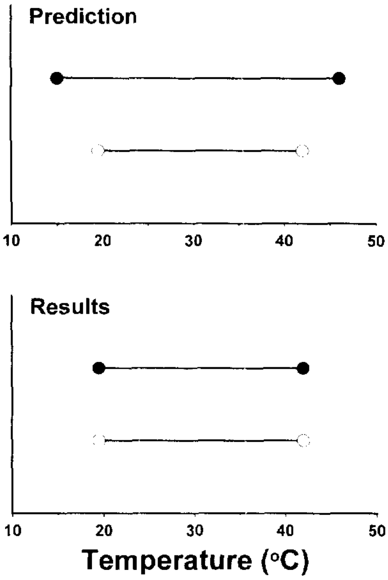

FIG. 3. Predicted and actual changes in thermal niche during the evolution of the thermal generalist group. Mean values of the ancestral niche limits are reported as open circles; those of the 32–42°C group, as filled circles. Contrary to prediction, the limits of the thermal niche did not increase beyond ancestral values. Data from Bennett and Lenski (1993).

Testing hypotheses: Resistance to stress will increase

Here we, along with other authors, utilize a definition of stress as a physical environmental factor that causes a substantial reduction in fitness (Sibly and Calow, 1989; Hoffmann and Parsons, 1991; Lenski and Bennett, 1993). Variable environments may include the possibility of encountering stressful or damaging conditions. In partic-

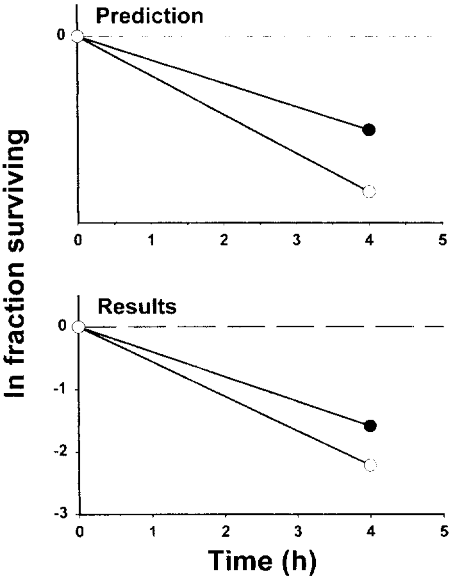

FIG. 4. Predicted and actual changes in stress tolerance during evolution of the thermal generalist group. Mean values for death rates at 50°C of the ancestor (open circles) and 32–42°C group (filled circles) are reported. As predicted, death rate declined significantly, indicating an increase in stress tolerance of the thermal generalist group. Unpublished data of Bennett and Lenski.

ular, the 32–42°C environment, half of which is poised within 1°C of the upper niche limit of the population, might be expected to entail the evolution of increased tolerance to thermal stress.

Resistance to thermal stress can be assayed as survival time in lethal thermal environments (Leroi *et al.*, 1994*a*). To examine the evolution of the stress response in the 32–42°C group, we allowed 6 replicate cultures of each of the 6 derived lines of this group, and 6 paired cultures of the ancestor, to grow for one day at 41°C, a temperature that activates formation of protective heat shock proteins in this organism (Gross, 1996). The paired derived and ancestral populations were then mixed and transferred to 50°C, a lethal temperature. For each type, we calculated its death rate over the first 4 hr of exposure to 50°C as the natural logarithm of the proportion of organisms that remained viable (Bennett and Lenski, unpub. data) (data reported in Fig. 4). For example, if 10% remained alive after 4 hr death rate would be −2.30 (ln 0.1). The difference between the death rate of the 32–42°C line and its paired ancestor was then used to express the evolutionary changes in stress tolerance. A positive value in this difference indicates a reduction in the death rate and thus an increase in thermotolerance. The mean change in the death rate for the 6 lines of the 32–42°C group was 0.595 (±0.375 95% C.L.), indicating a significant improvement ($P = 0.0095$ based on t-test) in stress resistance. This change corresponds to a nearly 50% reduction in the ancestral death rate during the 4 hr at 50°C (Fig. 4). *Hypothesis supported.* Evidently, the populations of the 32–42°C group can survive extreme temperatures better than their specialist ancestor, even thought they are unable to grow sufficiently to replace themselves at any higher temperature (which is reflected in the constancy of their thermal niche).

Testing hypotheses: Phenotypic plasticity will increase

Because of the frequent changes in temperature inherent in a variable thermal environment, it may be anticipated that populations evolving in such environments will become more adept at making those transitions and that this phenotypic plasticity will be an important component of their adaptation (Levins, 1968; Feder, 1978; Tsuji, 1988; see also general references West-Eberhard, 1989; Scheiner, 1993). It is possible to partition adaptation to a variable environment into its constant and transitional components, and to test whether either or both of them improved during evolutionary adaptation (Leroi *et al.*, 1994*b*). As discussed above, the 32–42°C group did undergo significant adaptation to both of the constant components (32°C and 42°C) of its environment. Adaptation to the transitional components may be analyzed by subtracting fitnesses of lines previously acclimated to competition temperatures from fitnesses of lines previously acclimated to another temperature (Leroi *et al.*, 1994*b*). In this case, the sum of W of a line acclimated and competed at 32°C and W acclimated and competed at 42°C is subtracted from the sum of W of the line acclimated at 32°C and

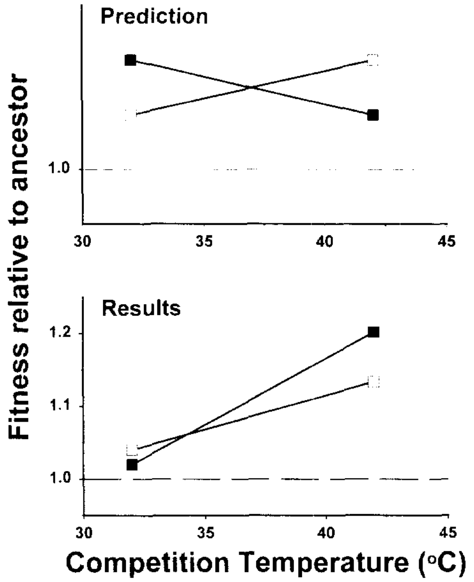

FIG. 5. Predicted and actual changes in phenotypic plasticity during evolution of the thermal generalist group. Open squares indicate mean fitness (relative to the ancestor) of the 6 lines of the 32–42°C group acclimated to 32°C; closed squares, acclimated to 42°C. If phenotypic plasticity had improved, then fitness relative to the ancestor would have increased more in the transitional environments (42°C acclimation, 32°C competition; 32°C acclimation, 42°C competition) than in the constant environments (acclimation and competition at the same temperature). The opposite pattern was observed: fitness was greater in the constant than in the transitional environments. Data from Leroi *et al.*, 1994*b*.

competed at 42°C and acclimated at 42°C and competed at 32°C. A positive value of this fitness differential would indicate that the transitional component had improved during evolution in the variable environment (Fig. 5). The mean of this fitness differential for the 6 lines of the 32–42°C group is −0.088 (±0.098 95% C.L.), a value that is marginally significant but in the direction opposite to that predicted (P = 0.07 based on t-test; P = 0.03 based on Wilcoxon signed rank test). Whether there has been a real decline in performance during thermal transitions is statistically debatable, but it is clear that adaptation to the variable environment did not lead to improvement specifically in the transitional components of fitness. *Hypothesis not supported.*

Implications for the evolution of thermal generalists and specialists

We used data from this experimental system to test general predictions concerning evolution in variable environments. Some of these evolutionary predictions were supported by our results. Specifically, fitness and efficiency of resource use in the novel variable environment (and its constant components) increased, as did stress resistance. These improvements in performance accord well with widespread views concerning adaptation and its correlates in variable environments. However, two other common predictions concerning anticipated expansion of function were not supported: neither niche breadth nor phenotypic plasticity increased during genetic adaptation to the variable environment. Thus, some of the anticipated adaptive patterns emerged, but not all of them. Further, and potentially more damaging for the general theory, the presumed tradeoffs did not emerge. The jack-of-all-trades became the master of all of them, too. Neither fitness nor efficiency declined at the constant ancestral temperature of 37°C; instead, both of them increased. In fact, the fitness at 37°C of the 32–42°C group increased even more than did that of the 37°C "control" group, which continued to evolve at this temperature throughout the second 2,000 generations (for the 37°C group, W = 1.025 ± 0.020 95% C.L., P = 0.036 based on t-test; Bennett and Lenski, 1996). Thus, the 32–42°C group gained functional capacities in the variable temperatures in which it had evolved without concomitantly losing capacity at the intermediate constant temperature to which its ancestor (and the 37°C control group) was adapted. In other words, the thermal generalist traded up instead of trading off.

What are the general implications of these results? If one asserts that these hypotheses describe *necessary* conditions of adaptation to a variable environment, then over half of them are refuted, because a single negative example is sufficient to reject such a hypothesis. If instead it is asserted that these hypotheses are usually but not invariably true, then the question remains open as to the degree of their applicability. Only investigations on other biological taxa can reveal their generality. Perhaps this system will be the only counterexample ever discovered. However, we believe that some skepticism is warranted with respect to the hypotheses' generality if the first such detailed investigation yields so many negative results. A conservative approach would dictate that much of the current theory dealing with evolution in variable or changing environments, particularly that involving assumptions of tradeoffs, needs to be revisited and revised.

The power and utility of laboratory selection studies

The preceding study illustrates how evolutionary physiological studies using laboratory selection can be used to test general theory. Such studies can help us understand the limits to the predictability of evolutionary change in physiological characters. They also can be used to test further the generality of the results obtained. For example, one might take the view that the daily alteration between 32°C and 42°C was not the most appropriate example of a variable thermal environment in which to test these hypotheses. Perhaps an environment with a temperature that changed continuously or randomly would have produced adaptive patterns more in accord with theory. The power of this approach is that such experiments could be designed and executed without undue difficulty to test those very propositions. Thus, like any good study, additional research to address more specific questions is suggested by the results. Furthermore, the results may inspire similar investigations, in either laboratory or natural systems, to test old or new hypotheses in other taxa and in other contexts. The generality of adaptive patterns, both expected and unexpected, will eventually be elucidated through comparative studies. The power and utility of laboratory selection studies is that one can clearly formulate and test assumptions and predictions about environmental adaptation in well-controlled systems. The results of these studies can then point the way for further investigations.

ACKNOWLEDGMENTS

Our research results reported here were supported by NSF Grants DEB-9208662 and IBN-9507416. We thank P. McDonald for technical assistance and A. Cullum, J. Hicks, S. O'Steen, and M. Riehle for helpful suggestions on the manuscript.

REFERENCES

Arnold, S. J. 1987. Genetic correlation and the evolution of physiology. *In* M. E. Feder, A. F. Bennett, W. Burggren, and R. B. Huey (eds.), *New directions in ecological physiology*, pp. 189–215. Cambridge University Press.

Barnes, P. T. and C. C. Laurie-Ahlberg. 1986. Genetic variability of flight metabolism in *Drosophila melanogaster*. III. Effects of GPDH allozymes and environmental temperature on power output. Genetics 113:267–294.

Bell, G. 1997*a*. *Selection: The mechanism of evolution*. Chapman and Hall, New York.

Bell, G. 1997*b*. *The basics of selection*. Chapman and Hall, New York.

Bennett, A. F. 1987. Inter-individual variability: An underutilized resource. *In* M. E. Feder, A. F. Bennett, W. Burggren, and R. B. Huey (eds). *New directions in ecological physiology*, pp. 147–169. Cambridge University Press, Cambridge.

Bennett, A. F. 1997. Adaptation and the evolution of physiological characters. *In* W. H. Dantzler (ed.), *Handbook of comparative physiology*, Vol. 1, pp. 3–16. Oxford University Press, New York.

Bennett, A. F. and R. B. Huey. 1990. Studying the evolution of physiological performance. Oxford Surveys Evol. Biol. 7:251–284.

Bennett, A. F. and R. E. Lenski. 1993. Evolutionary adaptation to temperature. II. Thermal niches of experimental lines of *Escherichia coli*. Evolution 47:1–12.

Bennett, A. F. and R. E. Lenski. 1996. Evolutionary adaptation to temperature. V. Adaptive mechanisms and correlated responses in experimental lines of *Escherichia coli*. Evolution 50:493–503.

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

Bennett, A. F. and J. A. Ruben. 1979. Endothermy and activity in vertebrates. Science 206:649–654.

Bennett, A. F. and J. A. Ruben. 1986. The metabolic and thermoregulatory status of therapsids. *In* N. Hotton, P. D. MacLean, J. J. Roth, and E. C. Roth (eds.). *The ecology and biology of mammal-like reptiles*, pp. 207–218. Smithsonian Institution, Washington, D.C.

Block, B. A., J. R. Finnerty, A. F. R. Stewart, and J. Kidd. 1993. Evolution of endothermy in fish: Mapping physiological traits on a molecular phylogeny. Science 260:210–214.

Buchmiller, T. L., K. S. Shaw, H. L. Chopourian, K. C. Lloyd, J. P. Gregg, F. A. Rivera, Jr., M. L. Lam, J. M. Diamond, and E. W. Fonkalsrud. 1993. Effect of transamniotic administration of epidermal growth factor on fetal rabbit small intestinal nutrient transport and disaccharidase development. J. Ped. Surg. 28:1239–1244.

Bumpus, H. 1899. The elimination of the unfit as illustrated by the introduced sparrow, *Passer domesticus*. Mar. Biol. Lab. (Woods Hole), Biol. Lect. (1898), p. 209–228.

Chippindale, A. K., D. T. Hoang, P. M. Service, and M. R. Rose. 1994. The evolution of development in *Drosophila melanogaster* selected for postponed senescence. Evolution 48:1880–1899.

Cossins, A. R. and K. Bowler. 1987. *Temperature biology of animals*. Chapman and Hall, London.

Dallinger, W. H. 1887. The president's address. J. Royal Microscop. Soc. April issue, pp. 185–199.

Denby, D. 1997. In Darwin's wake. New Yorker 73: 50–62.

Diamond, J. M. 1992. Evolutionary physiology—the red flag of optimality. Nature 355:204–206.

Diamond, J. M. 1993. Evolutionary physiology—quantitative design of life. Nature 366:405–406.

DiMichele, L., K. T. Paynter, and D. A. Powers. 1991. Evidence of lactate dehydrogenase-B allozyme effects in the teleost, *Fundulus heteroclitus*. Science 253:898–900.

DiMichele, L. and D. A. Powers. 1982. Physiological basis for swimming endurance differences between LDH-B genotypes of *Fundulus heteroclitus*. Science 216:1014–1016.

Djawdan, M., T. Sugiyama, L. Schlaeger, T. J. Bradley, and M. R. Rose. 1996. Metabolic aspects of the trade-off between fecundity and longevity in *Drosophila melanogaster*. Physiol. Zool. 69:1175–1195.

Dohm, M. R., J. P. Hayes, and T. Garland, Jr. 1996. Quantitative genetics of sprint running speed and swimming endurance in laboratory house mice (*Mus domesticus*). Evolution 50:1688–1701.

Dykhuizen, D. 1978. Selection for tryptophan auxotrophs of *Escherichia coli* in glucose-limited chemostats as a test of the energy conservation hypothesis of evolution. Evolution 32:125–150.

Dykhuizen, D. E. and A. M. Dean. 1990. Enzyme activity and fitness: Evolution in solution. Trends Ecol. Evol. 5:257–262.

Elena, S. F. and R. E. Lenski. 1997. Test of synergistic interactions among deleterious mutations in bacteria. Nature 390:395–398.

Endler, J. A. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34:76–91.

Endler, J. A. 1983. Natural selection on color patterns in poeciliid fishes. Environ. Biol. Fishes 9:173–190.

Endler, J. A. 1986. *Natural selection in the wild*. Princeton University Press, Princeton, New Jersey.

Falconer, D. S. 1996. *Introduction to quantitative genetics*. 4th ed. Longman Press, London.

Feder, M. E. 1978. Environmental variability and thermal acclimation in neotropical and temperate zone salamanders. Physiol. Zool. 51:7–16.

Feder, M. E., A. F. Bennett, W. Burggren, and R. B. Huey. 1987. *New directions in ecological physiology*. Cambridge University Press.

Feder, M. E., N. V. Cartano, L. Milos, R. A. Krebs, and S. L. Lindquist. 1996. Effect of engineering HSP70 copy number on HSP70 expression and tolerance of ecologically relevant heat shock in larvae and pupae of *Drosophila melanogaster*. J. exp. Biol. 199:1837–1844.

Felsenstein, J. 1985. Phylogenies and the comparative method. Am. Nat. 125:1–15.

Fields, P. A. and G. N. Somero. 1997. Amino acid sequence differences cannot fully explain interspecific variation in thermal sensitivities of gobiid fish A_4-lactate dehydrogenases (A_4-LDHs). J. exp. Biol. 200:1839–1850.

Futuyma, D. J. 1998. *Evolutionary biology*. 3rd ed. Sinauer Assoc., Sunderland, Massachusetts.

Futuyma, D. J. and G. Moreno. 1988. The evolution of ecological specialization. Ann. Rev. Ecol. Syst. 19:207–233.

Garland, T., Jr. 1999. An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. Amer. Zool., 39:000–000.

Garland, T., Jr. and S. C. Adolph. 1994. Why not to do two-species comparative studies—limitations on inferring adaptation. Physiol. Zool. 67:797–828.

Garland, T., Jr. and A. F. Bennett. 1990. Genetic basis of activity metabolism. 2. Quantitative genetics of maximal oxygen consumption in a garter snake. Am. J. Physiol. 259:R986–R992.

Garland, T., Jr., A. F. Bennett, and C. B. Daniels. 1990. Heritability of locomotor performance and its correlates in a natural population. Experientia 46: 530–533.

Garland, T., Jr. and P. A. Carter. 1994. Evolutionary physiology. Ann. Rev. Physiol. 56:579–621.

Garland, T., Jr., K. L. M. Martin, and R. Diaz-Uriarte. 1997. Reconstructing ancestral trait values using squared-change parsimony: Plasma osmolarity at the origin of amniotes. *In* S. S. Sumida and K. L. M. Martin (eds.), *Amniote origins: Completing the transition to land*, pp. 425–501. Academic Press, San Diego California.

Gibbs, A. G., A. K. Chippindale, and M. R. Rose. 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. J. exp. Biol. 200:1821–1832.

Gilchrist, G. W. 1995. Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. Am. Nat. 146:252–270.

Gould, S. J. and R. C. Lewontin. 1979. The spandrels of San Marco and the Panglossian paradigm: A critique of the adaptationist programme. Proc. Roy. Soc. London, Ser. B 205:581–598.

Grant, B. R. and P. R. Grant. 1993. Evolution of Darwin's finches caused by a rare climatic event. Proc. Roy. Soc. London Ser. B 251:111–117.

Grant, P. R., B. R. Grant, J. N. M. Smith, I. J. Abbott, and L. K. Abbot. 1976. Darwin's finches: Population variation and natural selection. Proc. Nat. Acad. Sci. U.S.A. 13:257–261.

Graves, J. L., E. C. Toolson, C. Jeong, L. N. Vu, and

M. R. Rose. 1992. Desiccation, flight, glycogen, and postponed senescence in *Drosophila melanogaster*. Physiol. Zool. 65:268–286.

Gross, C. A. 1996. Function and regulation of heat shock proteins. *In* F. C. Neidhardt (ed.), *Escherichia coli and Salmonella: Cellular and molecular biology*. 2nd ed., pp. 1382–1399. American Society for Microbiology, Washington, D.C.

Hammond, K. A., M. Lam, K. C. Lloyd, and J. Diamond. 1996*b*. Simultaneous manipulation of intestinal capacities and nutrient loads in mice. Am J Physiol, 271:G969–G979.

Hammond, K. A., K. C. Lloyd, and J. Diamond. 1996*a*. Is mammary output capacity limiting to lactational performance in mice? J. exp. Biol. 199: 337–349.

Harold, F. M. 1986. *The vital force: A study of bioenergetics*. W. H. Freeman and Co., New York.

Harvey, P. H. and M. D. Pagel. 1991. *The comparative method in evolutionary biology*. Oxford University Press, Oxford.

Heinrich, B. 1996. *The thermal warriors*. Harvard University Press.

Hochachka, P. 1997. Is evolutionary physiology useful to mechanistic physiology? The diving response in pinnipeds as a test case. Zool.—Anal. Complex Syst. 100:328–335.

Hoffmann, A. A. and P. A. Parsons. 1989. An integrated approach to environmental stress tolerance and life-history variation: Dessication tolerance in *Drosophila*. Biol. J. Linn. Soc. 37:117–136.

Hoffmann, A. A. and P. A. Parsons. 1991. *Evolutionary genetics and environmental stress*. Oxford University Press, Oxford.

Holland, L. Z., M. McFall-Ngai, and G. N. Somero. 1997. Evolution of lactate dehydrogenase-A homologs of barracuda fishes (genus *Sphyraena*) from different thermal environments: Differences in kinetic properties and thermal stability are due to amino acid substitutions outside the active site. Biochemistry 36:3207–3215.

Huey, R. B., D. Berrigan, G. W. Gelchrist, and J. C. Herron. 1999. Testing the "Beneficial Acclimation Hypothesis." Am. Zool., 39:000–000.

Huey, R. B. and D. Berrigan. 1996. Testing evolutionary hypotheses of acclimation. *In* I. A. Johnston and A. F. Bennett (eds.), *Animals and temperature: Phenotypic and evolutionary adaptation*, pp. 205–237. Cambridge University Press.

Huey, R. B., W. D. Crill, J. G. Kingsolver, and K. E. Weber. 1992. A method for rapid measurement of heat or cold resistance of small insects. Funct. Ecol. 6:489–494.

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

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

Jayne, B. C. and A. F. Bennett. 1990. Selection on locomotor performance capacity in a natural population of garter snakes. Evolution 44:1204–1229.

Kingsolver, J. G. 1995. Fitness consequences of seasonal polyphenism in western white butterflies. Evolution 49:942–954.

Kingsolver, J. G. 1996. Experimental manipulation of wing pigment pattern and survival in western white butterflies. Am. Nat. 147:296–306.

Koehn, R. K. 1987. The importance of genetics to physiological ecology. *In* M. E. Feder, A. F. Bennett, W. Burggren, and R. B. Huey (eds.), *New directions in ecological physiology*, pp. 170–185. Cambridge University Press.

Koehn, R. K., R. I. E. Newell, and F. W. Immermann. 1980. Maintenance of an aminopeptidase allele frequency cline by natural selection. Proc. Nat. Acad. Sci., U.S.A. 77:5385–5389.

Koehn, R. K., A. J. Zera, and J. G. Hall. 1983. Enzyme polymorphism and natural selection. *In* M. Nei and R. K. Koehn (eds.), *Evolution of genes and proteins*, pp. 115–136. Sinauer Associates, Sunderland, Massachusetts.

Lauder, G. V. 1981. Form and function: Structural analysis in evolutionary morphology. Paleobiology 7:430–442.

Lenski, R. E. and A. F. Bennett. 1993. Evolutionary response of *Escherichia coli* to thermal stress. Am. Nat. 142:S47–S64.

Lenski, R. E., M. R. Rose, S. C. Simpson, and S. C. Tadler. 1991. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. Am. Nat. 138:1315–1341.

Lenski, R. E., V. Souza, L. P. Duong, Q. G. Phan, T. N. M. Nguyen, and K. P. Bertrand. 1994. Epistatic effects of promoter and repressor functions of the Tn*10* tetracycline-resistance operon on the fitness of *Escherichia coli*. *Molec. Ecol.* 3:127–135.

Leroi, A. M., A. F. Bennett, and R. E. Lenski. 1994*a*. Temperature acclimation and competitive fitness: An experimental test of the Beneficial Acclimation Assumption. Proc. Nat. Acad. Sci. U.S.A. 91: 1917–1921.

Leroi, A. M., R. E. Lenski, and A. F. Bennett. 1994*b*. Evolutionary adaptation to temperature. III. Adaptation of *Escherichia coli* to a temporally varying environment. Evolution 48:1222–1229.

Levins, R. 1968. *Evolution in changing environments*. Princeton University Press, Princeton.

Loeschcke, V. and R. A. Krebs. 1996. Selection for heat-shock resistance in larval and in adult *Drosophila buzzatii*: Comparing direct and indirect responses. Evolution 50:2354–2359.

Losos, J. B. 1990. Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: An evolutionary analysis. Ecol. Monogr. 60:369–388.

Lynch, M. and B. Walsh. 1998. *Genetics and analysis of quantitative traits*. Sinauer Associates, Sunderland, Massachusetts.

McColl, G., A. A. Hoffmann, and S. W. McKechnie. 1996. Response of two heat shock genes to selection for knockdown heat resistance in *Drosophila melanogaster*. Genetics 143:1615–1627.

Miles, D. B. 1987. Habitat related differences in locomotion and morphology in two populations of *Urosaurus ornatus*. Am. Zool. 27:44A.

Mitton, J. B. 1994. Molecular approaches to population biology. Ann. Rev. Ecol. Syst. 25:45–69.

Mongold, J. A., A. F. Bennett, and R. E. Lenski. 1996*a*. Evolutionary adaptation to temperature. IV. Adaptation of *Escherichia coli* at a niche boundary. Evolution 50:35–43.

Mongold, J. A., A. F. Bennett, and R. E. Lenski. 1996*b*. Experimental investigations of evolutionary temperature adaptation. *In* I. A. Johnston and A. F. Bennett (eds.), *Animals and temperature: Phenotypic and evolutionary adaptation*, pp. 239–264. Cambridge University Press.

Mukai, T. 1969. The genetic structure of natural populations of *Drosophila melanogaster*. VII. Synergistic interaction of spontaneous mutant polygenes controlling viability. Genetics 61:749–761.

Nguyen, T. N. M., Q. G. Phan, L. P. Duong, K. P. Bertrand, and R. E. Lenski. 1989. Effects of carriage and expression of the Tn*10* tetracycline resistance operon on the fitness of *Escherichia coli* K12. Molec. Biol. Evol. 6:213–225.

Partridge, L., B. Barrie, N. H. Barton, K. Fowler, and V. French. 1995. Rapid laboratory evolution of adult life-history traits in *Drosophila melanogaster* in response to temperature. Evolution 49:538–544.

Pierce, V. A. and D. L. Crawford. 1997. Phylogenetic analysis of glycolytic enzyme expression. Science 276:256–259.

Powers, D. A. 1987. A multidisciplinary approach to the study of genetic variation within species. *In* M. E. Feder, A. F. Bennett, W. Burggren, and R. B. Huey (eds.), *New directions in ecological physiology*, pp. 102–130. Cambridge University Press, Cambridge.

Prosser, C. L. 1991. Temperature. *In* C. L. Prosser (ed.), *Environmental and metabolic animal physiology* (*Comparative animal physiology*, 4th ed.), pp. 109–165. Wiley-Liss Publishers, New York.

Reznick, D. N., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural population. Nature 346:357–359.

Reznick, D. and J. A. Endler. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution 36:160–177.

Rose, M. R. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution 38:1004–1010.

Rose, M. R., J. L. Graves, and E. W. Hutchison. 1990. The use of selection to probe patterns of pleiotropy in fitness characters. *In* F. Gilbert (ed.), *Insect life cycles: Genetics, evolution and co-ordination*, pp. 29–42. Springer Verlag, New York.

Ruben, J. A. and A. F. Bennett. 1980. The vertebrate pattern of activity metabolism: Its antiquity and possible relation to vertebrate origins. Nature 286: 886–888.

Scheiner, S. M. 1993. Genetics and evolution of phenotypic plasticity. Annu. Rev. Ecol. System. 24: 35–68.

Senez, J. C. 1962. Some considerations on the energetics of bacterial growth. Bacteriol. Rev. 26:95–107.

Service, P. M., E. W. Hutchinson, M. D. MacInley, and M. R. Rose. 1985. Resistance to environmental stress in *Drosophila melanogaster* selected for postponed senescence. Physiol. Zool. 58:380–389.

Sibly, R. M. and P. Calow. 1986. *Physiological ecology of animals: An evolutionary approach*. Blackwell Scientific Publications, Oxford.

Sinervo, B. 1990. The evolution of maternal investment in lizards—an experimental and comparative analysis of egg size and its effects on offspring performance. Evolution 44:279–294.

Sinervo, B. and A. L. Basolo. 1996. Testing adaptation using phenotypic manipulations. *In* M. R. Rose and G. V. Lauder (eds.), *Adaptation*, pp. 149–185. Academic Press, San Diego, California.

Sinervo, B., P. Doughty, R. B. Huey, and K. Zamudio. 1992. Allometric engineering—a causal analysis of natural selection of offspring size. Science 258: 1927–1930.

Sinervo, B. and R. B. Huey. 1990. Allometric engineering—an experimental test of the causes of interpopulational differences in performance. Science 248:1106–1109.

Snyder, L. R. G. 1981. Deer mouse hemoglobins: Is there genetic adaptation to high altitude? Bioscience 31:299–304.

Somero, G. N. 1995. Proteins and temperature. Ann Rev. Physiol. 57:43–68.

Sorci, G., J. G. Swallow, T. Garland, Jr., and J. Clobert. 1995. Quantitative genetics of locomotor speed and endurance in the lizard *Lacerta vivipara*. Physiol. Zool. 68:698–720.

Swallow, J. G., P. A. Carter, and T. Garland, Jr. 1998*b*. Artificial selection for increased wheel-running behavior in house mice. Behav. Genet. 28:227–237.

Swallow, J. G., T. Garland, Jr., P. A. Carter, W. Z. Zhan, and G. C. Sieck. 1998*a*. Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*). J. Appl. Physiol. 84:69–76.

Taylor, C. R. and E. R. Weibel. 1981. Design of the mammalian respiratory system. I. Problem and strategy. Respir. Physiol. 44:1–10.

Tsuji, J. S. 1988. Thermal acclimation of metabolism in *Sceloporus* lizards from different latitudes. Physiol. Zool. 61:241–253.

Tsuji, J. S., R. B. Huey, F. H. VanBerkum, T. Garland, Jr., and R. G. Shaw. 1989. Locomotor performance of hatchling fence lizards (*Sceloporus occidentalis*)—quantitative genetics and morphometric correlates. Evol. Ecol. 3:240–252.

Walton, B. M. 1993. Physiology and phylogeny—the evolution of locomotor energetics in hylid frogs. Am. Nat. 141:26–50.

Watt, W. B. 1977. Adaptation at specific loci. I. Natural selection on phosphoglucose isomerase of *Colias* butterflies: Biochemical and population aspects. Genetics 87:177–194.

Watt, W. B., R. C. Cassin, and M. S. Swan. 1983. Adaptation at specific loci. III. Field behavior and survivorship differences among *Colias* PGI genotypes are predictable from in vitro biochemistry. Genetics 103:725–739.

Watt, W. B., K. Donohue, and P. A. Carter. 1996. Adaptation at specific loci. VI. Divergence *vs.* parallelism of polymorphic allozymes in molecular function and fitness-component effects among *Colias* species (Lepidoptera, Pieridae). Molec. Biol. Evol. 13:699–709.

Weibel, E. R., C. R. Taylor, and H. Hoppeler. 1991. The concept of symmorphosis: A testable hypothesis of structure function relationship. Proc. Nat. Acad. Sci. U.S.A. 88:10357–10361.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. Annu. Rev. Ecol. System. 20:249–278.

Corresponding Editor: Todd Gleeson