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Differential Patterns of Gene Expression and Gene Complement in Laboratory-Evolved Lines of *E. coli*[1]

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SYNOPSIS. Laboratory selection experiments play a prominent role in understanding organismal adaptation. Although bacteria are not yet commonly used for such experiments, they are well suited for analyses of both the organismic and the genetic basis of adaptation. Bacteria can be maintained in large populations while occupying limited laboratory space, have short generation times, are well characterized physiologically, biochemically, and genetically, and are readily frozen and revived from the freezer. In addition, the genomes of many species are completely sequenced and knowledge of gene function is unparalleled. Here we review general aspects of selection experiments, the history of using selection experiments in combination with thermal biology and genomics, and highlight findings from six lines of *Escherichia coli* adapted to high temperature (41.5°C), including changes in organismal fitness, physiological performance, gene complement and gene expression. Our results are an example of the powerful insights that can be discovered by combining the tools and analyses of many biological disciplines including genomics, evolutionary biology, genetics, and evolutionary physiology.

INTRODUCTION

Selection experiments, although having a long history among evolutionary geneticists (Falconer, 1992; Falconer and Mackay, 1996) as well as plant and animal breeders, are relatively new to comparative and evolutionary physiologists. The recent adoption of such selection experiments by comparative physiologists coincided with the birth of a new field, ''evolutionary physiology,'' in the early 1990s (Garland and Carter, 1994). Evolutionary physiology has identified both selection experiments and phylogenetically-based species comparisons as hallmark approaches for the study of organismal adaptation (Feder *et al.*, 2000). Selection experiments can be performed on behavioral, physiological or morphological traits, with a further analysis of the adaptive process at organismal, physiological, biochemical and/or genetic levels (Gibbs, 1999; Bennett, 2003). Distinct advantages of this approach comprise (1) the ability to observe evolution in action under controlled conditions, making no assumptions about evolutionary time or relationships; (2) the maintenance of control populations over the same evolutionary time, permitting differentiation of adaptive responses from stochastic ones; (3) the use of replicate selected populations to reveal whether evolutionary adaptation repeatedly follows the same route to reach a given endpoint or whether alternative pathways lead to different endpoints. Analysis of the genetic bases of adaptation in selection experiments addresses the degree to which parallel changes at the level of phenotype are the result of parallel changes in genotype. This review will briefly consider the three main laboratory selection methods, the importance of experimental design and organismal choice, the history of using selected populations to address thermal adaptation, and the synergy of genomics and experimental evolution. We will focus on high temperature adaptation in *Escherichia coli* as a model for dissection of the genetic basis of adaptation.

Types of selection experiments

Laboratory selection experiments are typically performed via one of three methods: artificial truncation, laboratory culling, or laboratory natural selection (Rose *et al.*, 1990). All three methods work to modify genotype and phenotype in populations of interest, study evolution in action, and result in evolutionary histories that are known and controlled. However, there are major differences between these methods in terms of the identification of reproducing organisms and the severity of the stress applied.

Artificial truncation selection is a method familiar to many due to its pervasive use in plant and animal breeding. With this method, selection is imposed on a trait chosen by the experimenter prior to the start of the experiment. In each generation, this trait is assessed and only individuals in the top or bottom fraction of organisms possessing the trait are allowed to breed. Selection can be performed in either the positive (*e.g.*, increase in wing span) or negative (*e.g.*, decrease in wing span) direction, or in both directions in separate population (termed bi-directional selection). Artificial truncation selection methods have been used to select on behavioral, physiological, biochemical and morphological characters, including nest building behavior in mice (Bult and Lynch, 1997), voluntary running in mice (Swallow *et al.*, 1998), aerobic endurance in rats (Koch and Britton, 2001), juvenile hormone esterase activity in crickets (Zera and Zhang, 1995), and eye-spot size in butterflies (Beldade *et al.*, 2002).

The second method, laboratory culling selection in-

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volves exposing populations to a lethal environmental stress each generation. After a large proportion of the population has died, the surviving individuals are allowed to recover and breed in a favorable environment. No individual phenotypes are measured and breeding organisms are chosen based on their ability to survive a bout of lethal stress. Therefore, this method differs from artificial truncation selection as no trait is chosen *a priori*; rather individuals are free to arrive at whatever combinations of traits permit lethal stress survival. For example, this method has been used to select for dessication resistance and urea resistance in the fruit fly, *Drosophila melanogaster* (Chippindale *et al.*, 1996; Folk *et al.*, 2001).

The third method, laboratory natural selection, involves exposing populations to a sublethal environmental stress. All individuals are permitted to breed and intrapopulation competition alone determines which traits persist to the next generation. Simply, the environment is altered and nature is allowed to run its course. Like laboratory culling, this method differs from artificial truncation selection by not selecting on a particular character or trait. However, unlike laboratory culling, laboratory natural selection employs soft selection (*e.g.,* a sublethal environmental alteration) as opposed to hard selection. For example, laboratory natural selection has been used to study temperature adaptation in bacteria and viruses (Bennett *et al.*, 1992; Bull *et al.*, 1997) and nutrient limitation in yeast (Ferea *et al.*, 1999).

Design of selection experiments and organism choice

There are a number of important issues to consider in choosing a particular organism for laboratory selection experiments. We will only summarize these considerations here; a more extensive review is available elsewhere (Garland, 2003).

First, for practical reasons the organisms should have a relatively short generation time to allow differentiation between control and selected lines within a reasonable period. Second, the organisms should be easy to maintain and breed in the laboratory. Third, in addition to the selected lines, control lines should be maintained and lines of each group adequately replicated. Replication is essential to rule out founder effects and genetic drift and allow statistical analysis of results. In addition, replicate populations permit analysis of evolutionary trajectories, *e.g.,* whether evolution occurs repeatedly by the same pathway or via multiple adaptive solutions. Fourth, selected populations should be large in size because in small populations the stochastic effects of genetic drift may mask selection imposed by the investigator. Finally, before beginning any laboratory selection experiment, organisms should adapt to ancestral laboratory conditions so that adaptation to the laboratory environment does not occur concurrently with changes due to selective pressure. Careful thought and consideration of all these issues prior to choosing an organism should make the task of laboratory selection easier and more fruitful.

Bacteria: ideal organisms for experimental evolution

In addition to possessing the desired organismal characteristics of short generation time, small physical size, and large population sizes, additional features make bacteria a unique and ideal system for selection experiments (Elena and Lenski, 2003). First, bacteria such as *E. coli* are among the most well characterized organisms physiologically, genetically, and biochemically (Neidhardt *et al.*, 1996). Second, bacteria are prokaryotic, haploid, asexual organisms in which recombination does not occur. Therefore, the entire genome remains in complete linkage, simplifying the use of genetic markers (Elena and Lenski, 2003). Third, since populations can be established from a single clone, variation results from newly arising mutations rather than standing variation within the source population. Fourth, since bacteria can be repeatedly frozen and revived, the adaptive process can be easily assessed at multiple timepoints along the evolutionary trajectory. Fifth, measurements of organismal fitness are possible via direct competition between derived genotypes and archived ancestral ones. Finally—and perhaps most importantly—knowledge of the complete genome sequence and gene function facilitates dissection of the genetic bases of adaptation (Blattner *et al.*, 1997).

Experimental evolution and thermal biology

Temperature is the single environmental variable that most profoundly affects the worldwide distribution and abundance of organisms. Temperature significantly affects most biological processes and a voluminous literature is devoted to the intricate relationship between animals and their thermal environment (*e.g.,* Johnston and Bennett, 1996). Investigators have probed the evolutionary dynamics of thermal performance and fitness using the experimental evolution approach in organisms ranging from mice to viruses (Barnett *et al.*, 1975; Barnett and Dickson, 1984; Bull *et al.*, 1997), selecting on a wide range of traits, including knockdown resistance (Gilchrist and Huey, 1999), inducible thermotolerance (Feder *et al.*, 2002), and heat stress survival (Huey *et al.*, 1991). In microorganisms, experiments in temporally varying environments have tested the idea that thermal generalists will be ''jacks-of-all trades'' and ''masters-of-none'' (Leroi *et al.*, 1994). Lines evolved under either varying or constant temperature have been used to examine shifts in thermal niche and thermal optima (Bennett and Lenski, 1993) and to examine direct and correlated responses (Bennett *et al.*, 1992; Bennett and Lenski, 1996).

Experimental evolution and the genetic bases of adaptation

In addition to characterizing phenotypic responses during selection experiments, the wider accessibility of genomic technologies has facilitated studies of the genetic bases of adaptation. These are written through changes in DNA sequence in a number of different ways (Fig. 1), and any combination of these genetic

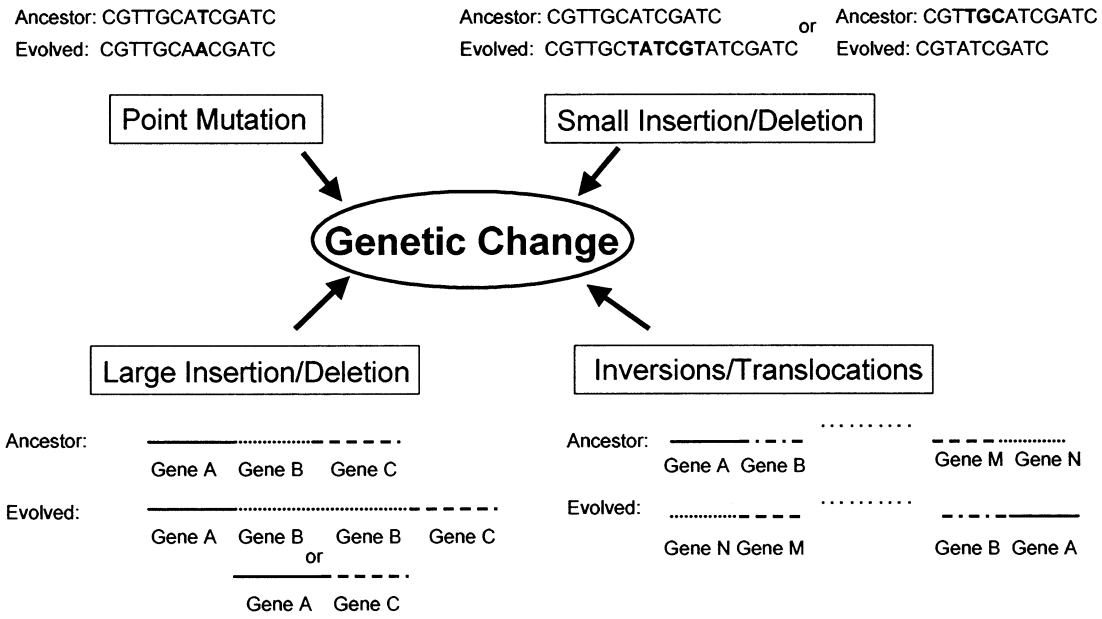

FIG. 1. Types of DNA Sequence Change as the Genetic Bases of Adaptation. These represent 4 general types of genetic changes that can underlie organismal adaptation.

changes can have profound effects on phenotype, from gene expression to organismal fitness. By obtaining information on the genetic architecture of evolution, we can address several questions regarding the adaptation process. What types of genetic changes occur? Are point mutations more or less likely than gene amplifications or deletions? How many events occur during the adaptive process? Do replicable changes at the phenotypic level result from parallel genetic changes, or do multiple independent genetic changes yield a convergent change in phenotype? However, to establish the relationship between genetic changes and adaptation firmly, two conditions must be met (1) a genetic change must be identified and (2) substitution of this mutation into the ancestral genetic background must yield a fitness improvement under selective conditions. Because of these stipulations, a comprehensive analysis of the genetic basis of adaptation has been possible to date only in small bacteriophage viruses with genomes (~5 kB) that can be completely sequenced, every mutation identified, and tested for a fitness effect. Only recently has DNA microarray technology been developed to aid discovery of genetic changes accompanying experimental evolution in more complex systems. DNA microarrays (Gracey and Cossins, 2003) can be exploited in analysis of selected populations to identify both evolutionary changes in gene complement and gene expression.

Experimental evolution and changes in gene expression

Since their introduction in the mid 1990s (Schena *et al.*, 1995), microarrays have primarily been used to make simultaneous measurements of transcript abundance for expressed genes in an organism. These genome-wide measurements of expression have typically concentrated on cataloguing responses to acute stress exposure and have only recently been exploited to track changes in gene expression occurring over evolutionary time (Ferea *et al.*, 1999; Cooper *et al.*, 2003; Riehle *et al.*, 2003). It is important to point out that transcriptome abundance is a phenotype and will reflect the cumulative effects of all genetic changes (Fig. 1) and that relatively few changes in genotype can result in extensive changes in phenotype. It is estimated that the number of beneficial mutations occurring over 2000 generations of adaptation in the bacterial system we employ is only 3 to 5 (Lenski and Travisano, 1994) and yet changes in the transcriptome number in the hundreds (Cooper *et al.*, 2003; Riehle *et al.*, 2003). A single genetic mutation in a regulatory gene can have profound effects on transcription of downstream genes and could potentially affect expression of an entire metabolic network; thereby generating the large number of transcriptome changes detected using microarrays.

Experimental evolution and changes in gene complement

One under-utilized power of microarray technology is the ability to use comparative genome hybridization to identify regions of the genome that have been amplified or deleted through evolutionary time (Riehle *et al.*, 2001; Dunham *et al.*, 2002). This technique is sensitive to identifying large insertion/deletion events, one of the flavors of genetic changes highlighted in Figure 1. To date this is the only flavor of DNA sequence variation for which adequate methods exists to identify genome-wide changes in organisms with large genome sizes.

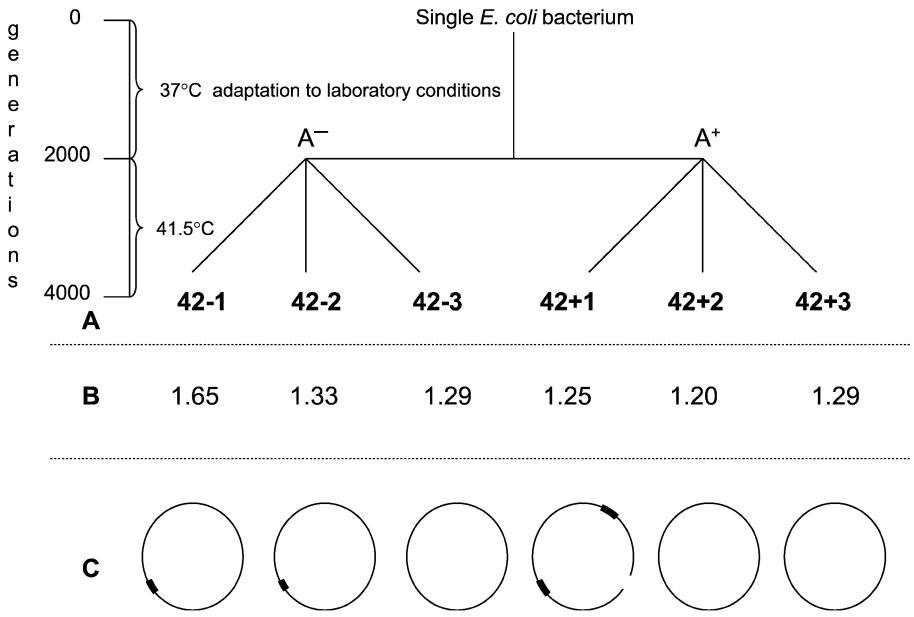

FIG 2. Fitness and Gene Complement in Six Thermally Derived *E. coli* Lines. **A.** The phylogeny of the selected lines. Lines began as a single *E. coli* bacterium that was grown under laboratory conditions (37°C in minimal media) for 2,000 generations to allow adaptation to the lab (Lenski *et al.*, 1991). After these first 2,000 generations, 2 ancestors were isolated and each gave rise to 3 high temperature derived lines that evolved for 2,000 generations at 41.5°C. **B.** Relative fitness at 41.5°C. All lines are significantly more fit than the ancestor ($P < 0.05$, Bennett and Lenski, 1996). **C.** Gene complement in thermally derived lines. Bolded regions indicated regions of gene amplification and missing regions indicate deletions (not to scale). Note the common duplicated region in 42−1, 42−2 and 42+1 (Riehle *et al.*, 2001).

A case study: thermal adaptation in Escherichia coli

Here we review our knowledge of the adaptation process occurring in response to stressful high temperature in *Escherichia coli*. This experimental evolution system began as a single bacterium that was introduced to the laboratory and allowed to adapt to laboratory conditions for 2,000 generations at 37°C in minimal medium (Lenski *et al.*, 1991; Fig. 2A). Noticeably, during this period of laboratory adaptation, fitness increased 35% relative to the ancestor, demonstrating that adaptation to the laboratory and the environmental stress condition need to be applied sequentially, not simultaneously. Then, two ancestors (A+ and A−) were isolated. Each ancestor gave rise to three independent high temperature lines, each grown for 2,000 generations at 41.5°C (Fig. 2A). For measurements of relative fitness, a high temperature line and the ancestor of the opposite genetic marker state (*e.g.*, A^- and 42+1, or A^+ and 42−1) are separately preconditioned to the competition environment, and then mixed in equal volumes in a competition flask and a sample is plated on indicator agar to determine starting densities. Due to the presence (A^+) or absence (A^-) of a neutral genetic marker (the ability or inability to utilize the sugar arabinose), ancestral and evolved colonies of opposite marker state are easily distinguished. The competition flask is then allowed to grow at an experimental temperature for 24 hr before a final sample is plated to determine differential reproduction in the ancestral and derived lines. Relative fitness is calculated as the ratio of derived to ancestral values (Lenski *et al.*, 1991); ratios greater than one indicate an increase in fitness in the derived line.

Changes in fitness and physiological performance

After 2,000 generations, all six lines showed significant increases in relative fitness at 41.5°C ranging between 20 and 65% (Bennett and Lenski, 1996; Fig. 2B). The rate of fitness increase in these high temperature evolved lines was higher than observed in control lines (only an additional 2% after another 2,000 generations at 37°C, Bennett *et al.*, 1992), such that significant fitness gains were obvious in only one month (200 generations, Bennett *et al.*, 1990). Further, these increases in fitness were not accompanied by tradeoffs in fitness under ancestral conditions (Bennett and Lenski, 1993), a condition also obtained in another high temperature selected microorganism (Shi and Xia, 2003). In addition, fitness at 20°C, the lower end of the thermal niche, was indistinguishable from that of the ancestor in five of the six lines (Bennett and Lenski, 1993). Despite selection very near their upper thermal maxima, only one line exhibited a change in its upper thermal maxima: 42−1 extended its upper thermal limit by 1°C and had decreased fitness at all temperatures below 37°C (Bennett and Lenski, 1993). The thermal niches of the other 5 lines remained 19°C to 42°C, indistinguishable from the ancestral thermal niche (Bennett and Lenski, 1993).

Changes in fitness at high temperature were accompanied by increases in yield (Cullum *et al.*, 2001) and growth rate (Riehle *et al.*, 2003). However, adaptation to high temperature did not confer cross-resistance (in-

creased fitness) to other novel stressors, including acid, alkali, ethanol, high osmolarity and peroxide (Cullum *et al.*, 2001). One of the correlated responses present in these lines was increased fitness in maltose at 41.5°C (Bennett and Lenski, 1996). Maltose is a glucose dimer having different transport mechanisms across both the inner and outer membrane, but once inside, is catabolized identically to glucose. Five of the six independently derived lines were significantly more fit in maltose than in glucose, despite at least 4,000 generations without exposure to maltose. Lastly, inducible thermotolerance, the ability to withstand a lethal stress (50°C) following pre-exposure to a hardening stress (41.5°C) was significantly greater in the high temperature lines as compared to their ancestors (Riehle *et al.*, 2003).

Changes in gene complement

In order to obtain information on evolved changes in gene complement via large insertion/deletion events (Fig. 1), genomic DNA was isolated from ancestral and derived lines, labeled radioactively, and hybridized onto a microarray containing all 4,290 genes in *E. coli*. Intensity differences in labeling were compared between the ancestral and high temperature lines to determine changes in gene copy number (Riehle *et al.*, 2001). Using this approach, we found 5 changes in gene complement occurring in 3 of the 6 high temperature evolved lines (42−1, 42−2, 42+1) (Riehle *et al.*, 2001; Fig. 2C). The other three lines had no large insertion/deletion events after 2,000 generations of growth at 41.5°C. Most interestingly, of the 5 changes in gene complement, 3 duplications occurred in an overlapping region of the bacterial chromosome in the three different lines, suggesting evolutionary replicability at the level of large insertion/deletion events. The region of common amplification (Fig. 2C) varied in size across the three lines, but the overlapping region contained 23 genes (10 of unknown function and 13 with functional characterization). None of the genes in this region were *a priori* candidate genes for thermal adaptation. However, at least four of these genes, *rpoS*, *sureE*, *nlpD*, and *pcm*, represent good *a posteriori* candidate genes, based on their previously established roles in stress and starvation survival (discussed in detail in Riehle *et al.*, 2001). Northern blotting analysis also showed that these genes were increased in expression in the lines with the amplified region (Riehle *et al.*, 2001). Expression in the lines without the amplified region was not increased, suggesting that these lines achieved increments in fitness via alternate genetic mechanisms. Thus far, our analysis indicates an association between this amplified genomic region and fitness at high temperature; however, establishment of causation requires future experimentation. Similar work on 8 strains of yeast evolved for 100–500 generations of growth in glucose-limited chemostats used very similar methods and also discovered replicable changes in gene complement, events unique to a single strain, strains completely void of major events and a role for transposable elements in changes in gene complement (Dunham *et al.*, 2002).

Changes in gene expression

DNA microarray technology is used primarily to measure whole genome transcript abundance. This experimental approach generates volumes of data, every replicate giving a single expression value for each individual gene (4,290 in the case of *E. coli*). In an effort to clarify results and focus our approach, we used a hypothesis testing approach. Using this approach, we identified a group of *a priori* candidate genes based on their established roles in acute thermal stress (Yura *et al.*, 2000). This group contained 35 genes whose protein products include molecular chaperones, ATP dependent proteases, protein folding catalysts, and members of the periplasmic stress response. To generate data to test the role of our candidate genes in the adaptation to high temperature, total RNA was isolated from the A^- ancestor and its three high temperature derived lines, reverse transcribed and labeled, and hybridized to a microarray. Derived and ancestral lines were compared gene by gene to determine evolved increments in expression, evolved decrements in expression, or no change in expression relative to the ancestor. We found that the group of thirty-five candidate genes showed significant evolutionary change in gene expression relative to the ancestor at 41.5°C (Riehle *et al.*, 2003). At the level of individual genes, changes in expression involved both increments and decrements relative to the ancestor, indicating that evolutionary adaptation to high temperature did not necessarily involve only increments in expression of candidate genes associated with acute thermal stress. Some changes occurred in parallel across evolutionary replicates, while others were highly heterogeneous (Riehle *et al.*, 2003). Lastly, in the three lines tested, only 1 of the 12 expression changes involved a molecular chaperone gene, while the extracytoplasmic stress response, occurring between the outer and inner membranes, appeared to be a hotbed of evolutionary change (Riehle *et al.*, 2003). We also found that the candidate genes were significantly more likely to show expression change than the non-candidate genes (the balance of the *E. coli* transcriptome expressed at 41.5°C). Overall, this approach established a role for these *a priori* candidate genes, known to play a role in acute thermal stress, in the long-term evolutionary response to thermal stress and highlights the utility of a hypothesis testing approach in wading through the mountains of data generated by expression profiling. The balance of expression data on non-candidate genes can be used in a complementary hypothesis generating approach.

CONCLUSIONS

Selection experiments represent a very powerful approach for the dissection of the adaptive process. Through a combination of laboratory natural selection and microarray technologies, we used high tempera-

ture selection experiments to address the replicability of adaptation at both phenotypic and genotypic levels in bacteria. Our results address the specifics of thermal adaptation in microorganisms, as well as general aspects of the evolutionary process. As microarray technology increasingly addresses the aims of evolutionary physiologists (Gracey and Cossins, 2003), the genetic architecture of adaptation will become defined in a wide array or organisms subjected to a range of selection pressures, resulting in characterization of the adaptive process from genotype to phenotype.

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REFERENCES

Barnett, S. A., K. M. H. Munro, J. L. Smart, and R. C. Stoddart. 1975. House mice bred for many generations in two environments. J. Zoology London 177:153–160.

Barnett, S. A. and R. G. Dickson. 1984. Changes among wild house mice (*Mus musculus*) bred for ten generations in a cold environment, and their evolutionary implications. J. Zoology London 203:163–180.

Beldade, P., K. Koops, and P. M. Brakefield. 2002. Developmental constraints versus flexibility in morphological evolution. Nature 416:844–847.

Bennett, A. F. 2003. Experimental evolution and the Krogh principle: Generating biological novelty for functional and genetic analyses. Physiol. Biochem. Zool. 76:1–11.

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

Bennett, A. F. and R. E. Lenski. 1993. Evolutionary adaptations 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.

Blattner, F. R., G. Plunkett, 3rd, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao. 1997. The complete genome sequence of *Escherichia coli* K-12. Science 277:1453–1474.

Bull, J. J., M. R. Badgett, H. A. Wichmann, J. P. Huelsenbeck, D. M. Hillis, A. Gulati, C. Ho, and I. J. Molineux. 1997. Exceptional convergent evolution in a virus. Genetics 147:1497–1507.

Bult, A. and C. D. Lynch. 1997. Nesting and fitness: Lifetime reproductive success in house mice bidirectionally selected for thermoregulatory nest-building behavior. Behav. Genet. 27: 231–240.

Chippindale, A. K., T. J. F. Chu, and M. R. Rose. 1996. Complex trade-offs and the evolution of starvation resistance in *D. melanogaster*. Evolution 50:752–766.

Cooper, T. F., D. E. Rozen, and R. E. Lenski. 2003. Parallel changes in gene expression after 20,000 generations of evolution in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 100:1072–1077.

Cullum, A. J., A. F. Bennett, and R. E. Lenski. 2001. Evolutionary adaptation to temperature. IX. Preadaptation to novel stressful environments of *Escherichia coli* adapted to high temperature. Evolution 55:2194–2202.

Dunham, M. J., H. Badrane, T. Ferea, J. Adams, P. O. Brown, F. Rosenzweig, and D. Botstein. 2002. Characteristic genome rearrangements in experimental evolution of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. 99:16144–16149.

Elena, S. F. and R. E. Lenski. 2003. Evolution experiments with microorganisms: The dynamics and genetic bases of adaptation. Nat. Rev. Genet. 4:457–469.

Falconer, D. S. 1992. Early selection experiments. Ann. Rev. Genet. 26:1–14.

Falconer, D. S. and T. F. C. Mackay. 1996. *Introduction to quantitative genetics*, 4th ed. Longman, Essex, England.

Feder, M. E., T. B. C. Bedford, D. R. Albright, and P. Michalak. 2002. Evolvability of HSP70 expression under artificial selection for inducible thermotolerance in independent populations of *Drosophila melanogaster*. Physiol. Biochem. Zool. 75:325–334.

Feder, M. E., A. F. Bennett, and Raymond B. Huey. 2000. Evolutionary physiology. Ann. Rev. Ecol. Sys. 31:315–341.

Ferea, T. L., D. Botstein, P. O. Brown, and R. F. Rosenzweig. 1999. Systematic changes in gene expression patterns following adaptive evolution in yeast. Proc. Natl. Acad. Sci. U.S.A. 96:9721–9726.

Folk, D. G., C. Han, and T. J. Bradley. 2001. Water acquisition and partitioning in *D. melanogaster*: Effects of selection for desiccation resistance. J. Exp. Biol. 204:3323–3331.

Garland, T., Jr. 2003. Selection experiments: An underutilized tool in biomechanics and organismal biology. *In* V. L. Bels, J.-P. Gasc, and A. Casinos (eds.), *Vertebrate biomechanics and evolution*, pp. 23–56. Bios. Scientific, Oxford.

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

Gibbs, A. 1999. Laboratory selection for the comparative physiologist. J. Exp. Biol. 202:2709–2718.

Gilchrist, G. W. and R. B. Huey. 1999. The direct response of *Drosophila melanogaster* to selection on knockdown temperature. Heredity 83:15–29.

Gracey, A. Y. and A. R. Cossins. 2003. Application of microarray technology in environmental and comparative physiology. Annu. Rev. Physiol. 65:231–259.

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

Johnston, I. A. and A. F. Bennett. 1996. *Animals and temperature: Phenotypic and evolutionary adapation*. Cambridge University Press, Cambridge, UK.

Koch, L. G. and S. L. Britton. 2001. Artificial selection for intrinsic aerobic endurance running in rates. Physiol. Genomics 5:45–52.

Lenski, R. E. and M. Travisano. 1994. Dynamics of adaptation and diversification: A 10,000 generation experiment with bacterial populations. Proc. Natl. Acad. Sci. U.S.A. 91:6808–6814.

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.

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

Neidhardt, F. C., R. Curtiss, III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger. 1996. *Escherichia coli and Salmonella*: *Cellular and Molecular Biology*. ASM Press, Washington D.C, USA.

Reznick, D. and J. Travis. 1996. The empirical study of adaptation in natural populations. *In* M. R. Rose and G. V. Lauder (eds.), Adaptation, pp. 243–289. Academic Press, San Diego.

Riehle, M. M., A. F. Bennett, and A. D. Long. 2001. Genetic architecture of thermal adaptation in Escherichia coli. Proc. Natl. Acad. Sci. U.S.A. 98:525–530.

Riehle, M. M., A. F. Bennett, R. E. Lenski, and A. D. Long. 2003. Evolutionary changes in heat inducible gene expression in lines of *Escherichia coli* adapted to high temperature. Physiol. Gen. 14:47–58.

Rose, M. R., J. L. Graves, and E. W. Hutchinson. 1990. The use of selection to probe patterns of pleiotropy in fitness characters. *In*

F. Gilbert (ed.), Insect life cycles: Genetics, evolution and coordination, pp. 29–42. Springer, New York.

Schena, M., D. Shalon, R. W. Davis, P. O. Brown. 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270:467–470.

Shi, B. and X. Xia. 2003. Changes in growth parameters of *Pseudomonas pseudoalcaligenes* after ten months culturing at increasing temperature. FEMS Micro. Ecol. 45:127–134.

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

Yura, T., M. Kanemori, and M. T. Morita. 2000. The heat shock response: Regulation and function. *In* G. Storz and R. Hengge-Aronis (eds.), Bacterial stress responses, pp. 1–18. ASM Press, Washington DC.

Zera, A. J. and C. Zhang. 1995. Evolutionary endocrinology of juvenile hormone esterase in *Gryllus assimilis*: Direct and correlated responses to selection. Genetics 141:1125–1134.