Chapter 2

Testing Antonovics' five tenets of ecological genetics: experiments with bacteria at the interface of ecology and genetics

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Introduction

When I began graduate school in 1977, I thought that ecology and genetics were completely distinct fields of study. I imagined that I could study ecological patterns and processes in blissful ignorance of genetics and without worrying that evolution would directly impinge on my research. This naïve view was soon dispelled by Janis Antonovics, who taught a wonderful course at Duke University in North Carolina on Ecological Genetics, which I took in 1979. In many treatments of population genetics, natural selection is largely devoid of its ecological context and appears only as an abstract coefficient, S, that operates on gene frequencies, p and q. But Antonovics' course placed selection squarely in its ecological context. Moreover, his course examined the ecological consequences of changing gene frequencies, thus emphasizing the feedback of evolutionary change on ecology.

Antonovics had synthesized his integrated view of ecology and genetics in a provocative paper published a few years earlier (Antonovics 1976). In that paper, he presented the following five tenets:

1 'The ecological amplitude of a species (both within and among communities) has a genetic component. Explaining the abundance and distribution of organisms is basically a genetic problem.' [p. 236]

2 'Forces maintaining species diversity and genetic diversity are similar. An understanding of community structure will come from considering how these kinds of diversity interact.' [p. 238]

3 'Darwinian fitness can be measured in terms of mortality and fecundity of individuals within populations. Adaptation is a dynamic process, operationally definable and not just a "matching" of the individual to the environment.' [p. 239]

4 'Genetic adjustment to environmentally induced changes in fecundity and mortality may be by direct response in the affected age-specific parameters or by compensatory change in other parts of the life history. Adaptation to new environments will result in different genotypes with different life histories.' [p. 240]

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5 'The distinction between "ecological time" and "evolutionary time" is artificial and misleading. Changes of both kinds may be on any time scale: frequently genetic and ecological changes are simultaneous.' [p. 241]

Antonovics (1976) offered evidence in support of these tenets based largely on studies of plant populations and communities. However, he also stated that the 'generality and usefulness of [the tenets] remain in need of assessment' [p. 236]. Thus, the tenets were intended not as dogma but rather to stimulate new lines of research. They have certainly done so for me. During the past two decades, my colleagues and I have conducted experiments with the bacterium *Escherichia coli* and sometimes its viral predators. The findings from these experiments provide an opportunity to re-examine the generality and utility of Antonovics' five tenets. (These tenets became known among students as the 'ecological geneticist's creed'. Over the years, some of the tenets have changed slightly and several new ones have been added, but in the interest of space I will restrict this paper to the five original tenets.)

The rest of this paper is organized as follows. Each of the next three sections summarizes a different set of experiments with *E. coli*, without explicit reference to Antonovics' tenets. Then I will return to the five tenets and discuss them in light of the experimental findings. I conclude by emphasizing the special importance of integrating ecology and genetics given today's rapid pace of environmental change.

Competition for a limiting resource

In one long-term project, we studied the dynamics of bacterial populations as they mutated and adapted by natural selection to a simple and constant environment (Lenski *et al.* 1991; Lenski & Travisano 1994). Twelve replicate populations were diluted daily into fresh medium in which glucose provided the sole source of energy; the populations were maintained at constant 37°C. Each day, the bacteria grew until they depleted the glucose, and the 1 : 100 dilution allowed about 6.6 generations of binary fission per day. This experiment has continued now for more than 20 000 bacterial generations (3000 days). At the start of the experiment and at intervals ever since, samples were stored in a freezer at –80°C, where the bacteria exist in a state of suspended animation but can be revived at any time for study. Thus, we have time series for all 12 populations of adaptation to the environment. We can even perform competition experiments between the derived bacteria and the ancestor using a neutral genetic marker that allows us to distinguish strains based on colour. Prior to their competition, both strains are acclimated to the environment in which they compete, in order that we can distinguish genetic adaptation from phenotypic acclimation.

Figure 2.1 shows the evolutionary trajectory for the mean competitive fitness of the derived bacteria relative to their common ancestor. Relative fitness is calculated simply as the ratio of realized growth rates of the derived and ancestral populations as they competed for limiting glucose (Lenski *et al.* 1991). Within a few hundred generations, the bacteria had already begun to improve appreciably, and by genera-

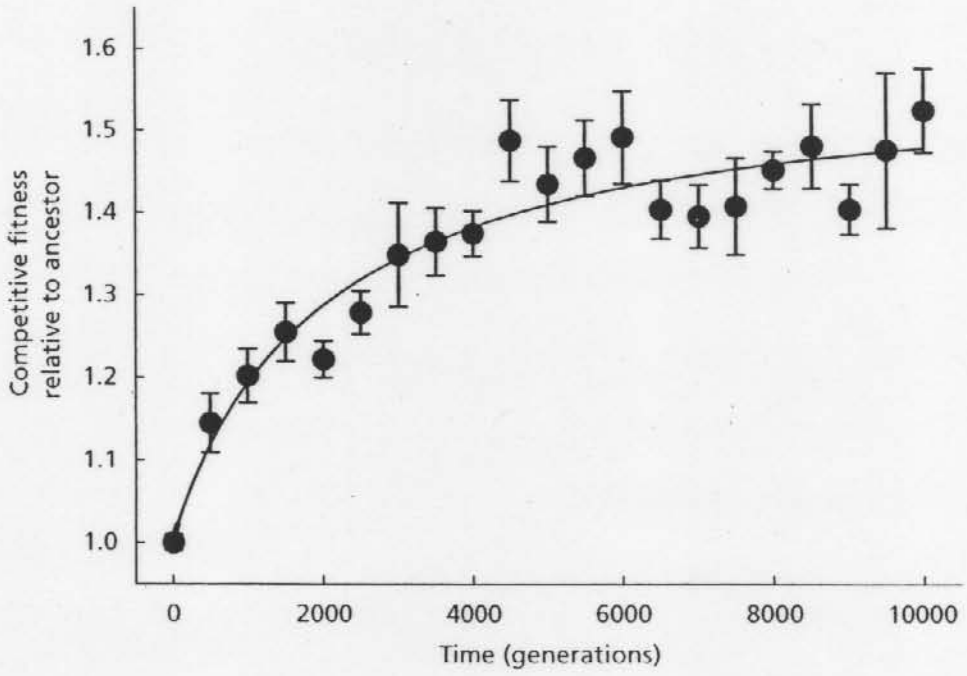

Figure 2.1 Trajectory for competitive fitness relative to the ancestor during 10 000 generations of experimental evolution in *E. coli*. Each point is the mean of 12 replicate populations; the error bars are 95% confidence limits. (Reproduced with permission from Lenski & Travisano (1994) [Copyright 1994 National Academy of Sciences, USA].)

tion 10 000 they were about 50% more fit, on average, then was the ancestor (Lenski & Travisano 1994). The competitiveness of these bacteria is not some fixed attribute of the species, but rather it mutates and evolves.

As the bacteria became better competitors for glucose, they also changed in other respects. One rather surprising change is that the density of bacteria that was reached each day, when the glucose was depleted, declined over time (Fig. 2.2). Evidently, greater fitness and higher population density do not necessarily correspond. Fitness is a dynamic measure that reflects relative rates of change in abundance; it should not be confused with measures of abundance per se. (This point is both obvious and reassuring when one considers that each of our intestines holds more bacteria than there are humans on this planet.) In the evolving populations, the average size of bacterial cells increased, as did the net volume of bacteria produced per unit of glucose (Fig. 2.2). Thus, the efficiency with which the bacteria converted resources into biomass increased (Lenski & Mongold 2000). Average cell size increased in all 12 replicate populations, but the reasons for this change remain unclear. In part, it reflects a positive phenotypic correlation between growth rate and cell size (such that selection for faster growth produces larger cell size, all else equal). However, this correlation cannot fully explain the observed increase in cell size, as the evolved bacteria produce larger cells even when they are forced to grow at the same rate as their ancestor (Mongold & Lenski 1996).

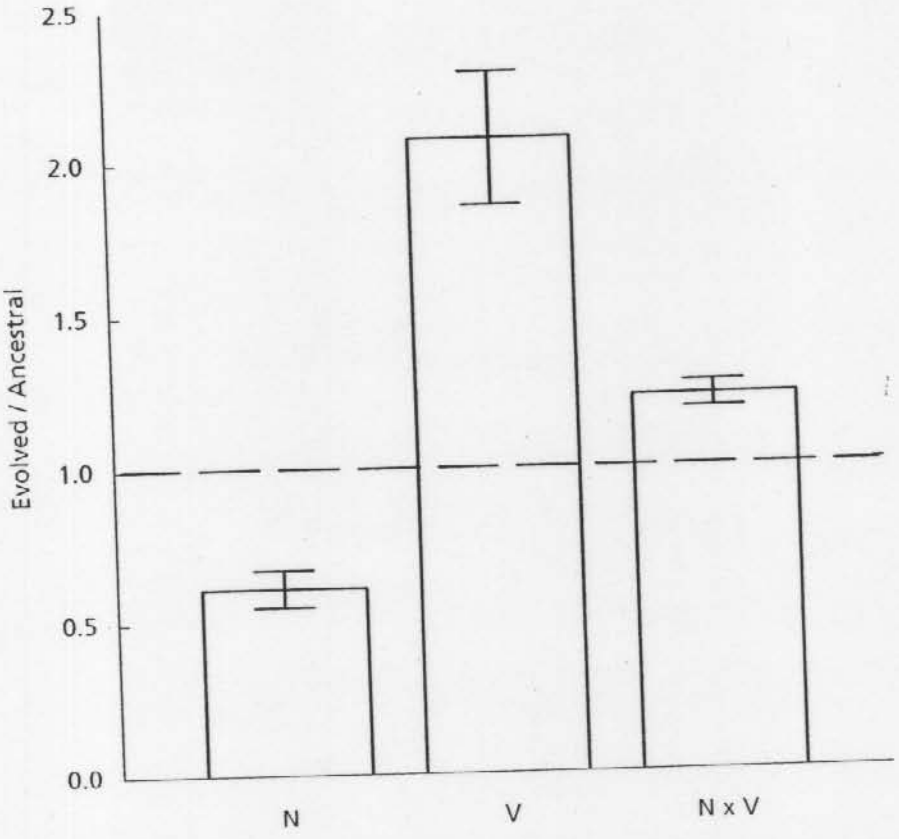

Figure 2.2 Changes in cell number (N), average cell volume (V), and total volume ($N \times V$) after 10 000 generations of evolution in *E. coli*. The height of each bar indicates the mean of 12 independently evolved populations relative to the ancestral value; the error bars are 95% confidence limits. (Data from Lenski & Travisano (1994).)

One does not usually think of bacteria like *E. coli* as having life histories: they seem simply to grow and divide. However, the concept of life history in ecology is not merely a description of morphologically distinct stages but implies an underlying demography that can be projected over time. Bacterial fitness depended on several distinct demographic parameters, even in the simple environment of the long-term experiment. Each day, the populations experienced 'seasons' of feast and famine: first a period of transition from starvation to growth after they were diluted in fresh medium, then a period of exponential growth, followed by another transition phase as the glucose was depleted, and finally a period of resource deprivation. One can ask therefore which life-history traits changed so as to yield the net improvement in competitive fitness (Vasi *et al.* 1994). The bacteria improved primarily in terms of a higher exponential growth rate and a shorter lag upon transfer into fresh medium (Fig. 2.3). However, there was no measurable change in their growth rate at low glucose concentration owing to an increase in K_s, which describes the concentration necessary to grow at half their maximal rate (Fig. 2.3). The bacteria also did not improve in their short-term survival after glucose was

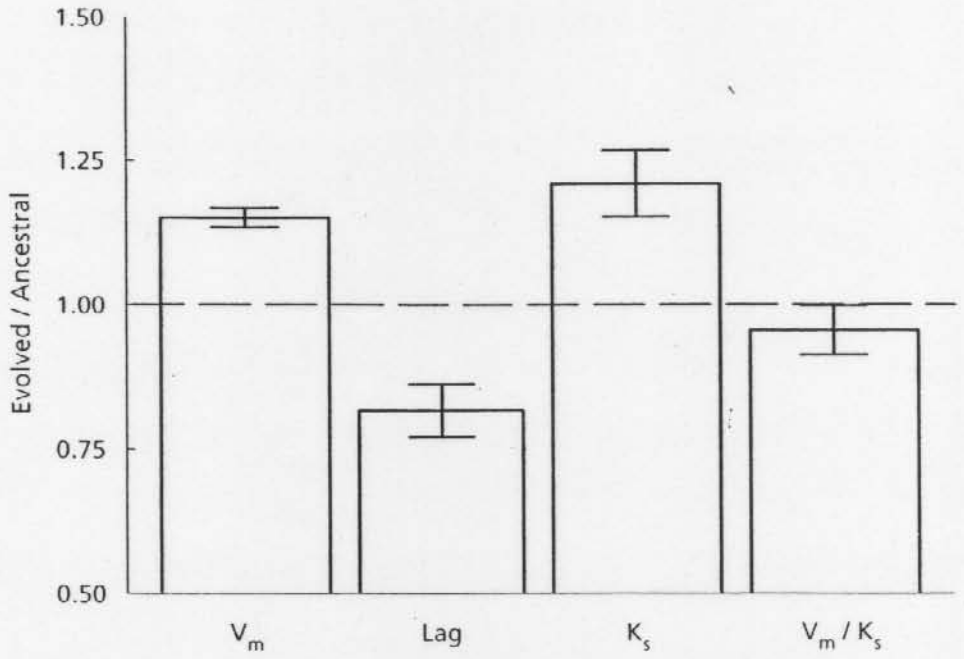

Figure 2.3 Changes in demographic parameters after 2000 generations of evolution in *E. coli*. V_m, maximum growth rate on glucose; Lag, duration of lag phase; K_s, concentration of glucose that supports a growth rate of $V_m/2$; V_m/K_s, proportional growth rate as glucose becomes extremely scarce. The height of each bar indicates the mean of 12 independently evolved populations relative to the ancestral value; the error bars are 95% confidence limits. (Data from Vasi *et al.* (1994).)

exhausted; mortality was not very important in the evolution experiment because the ancestor experienced no measurable mortality during starvation for less than a day. It must be emphasized that this pattern of life-history change depended on the selective environment. We and other groups have studied *E. coli* populations maintained under different conditions, including with low but steady resources and during prolonged starvation (Dykhuizen & Hartl 1981; Zambrano *et al.* 1993; Vasi & Lenski 1999). Such populations also adapted evolutionarily, but they did so by changing other demographic parameters that were more important in those environments.

Another aspect of this experiment that deserves mention is the opportunity for coexistence between ecologically distinct types. Each population was founded by a single genotype, glucose was the sole source of energy provided and limiting to population density, and the physical environment was homogeneous both spatially and temporally (except for daily resource fluctuations), all of which suggest little opportunity for niche diversification. Indeed, bacterial adaptations usually led to competitive exclusion of the previously dominant type—but not always. In one replicate, there evolved a community of two ecotypic 'species' (Rozen & Lenski 2000; see also Rosenzweig *et al.* 1994; Turner *et al.* 1996; Treves *et al.* 1998, for

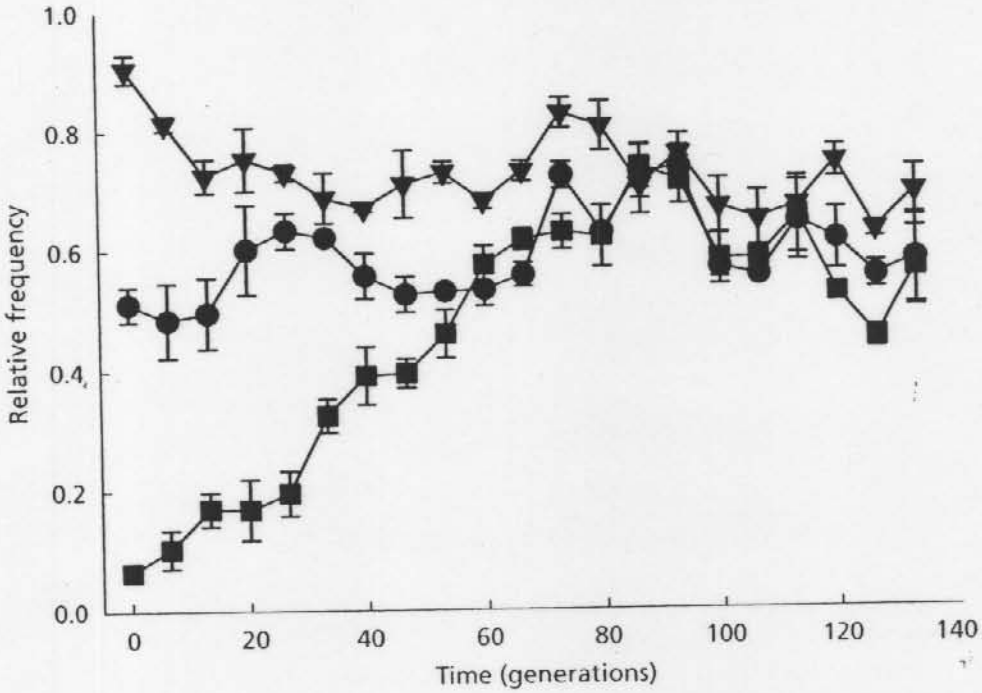

Figure 2.4 Stable equilibrium of two ecotypes that evolved within a population of *E. coli*. The two ecotypes were mixed at three initial ratios; they rapidly converged to an intermediate equilibrium. Each point is the mean of three replicate mixtures; the error bars are standard errors. (Reproduced with permission from Rozen & Lenski (2000).)

similar findings). The stability of their interaction was demonstrated by the fact that each ecotype, when introduced at low frequency, could invade the other type (Fig. 2.4). Based on their demographic parameters in pure culture, one ecotype was clearly superior at exploiting glucose. How did the other type persist in this simple regime? The superior exploiter of glucose secreted some metabolite into the medium that promoted the growth only of the other ecotype. The cross-feeding ecotype also appeared to inhibit the survival of the superior glucose competitor after the glucose was depleted. This two-member community emerged around generation 6000 and has persisted through generation 20 000 and beyond (Rozen & Lenski 2000). During that time, the numerical dominance of the two ecotypes has shifted back and forth several times. The cause of these reversals is not yet known, but a plausible explanation is that the 'ecological' equilibrium changed as each ecotype further adapted genetically to the physical environment or to its competitor. In any case, the organisms themselves have modified the environment such that two ecological strategies can coexist indefinitely.

Changing thermal environments

In the previous study, the populations faced an environment that was both benign and constant over the long-term. The bacteria became better competitors, but their

rate of improvement decelerated over time (see Fig. 2.1). In this next study, we took a clone from one of these populations that had adapted for 2000 generations to living on glucose at 37°C, and we used it to found 24 new bacterial populations that were propagated for 2000 more generations in the same medium, but under one of four different thermal regimes (Bennett *et al.* 1992). One regime was a continuation of the ancestral regime, and it served as a control for the absence of environmental change. Two other regimes were novel but constant environments, with temperature held at either 32°C or 42°C. The former is a benign temperature for *E. coli*; the bacteria grow somewhat slower than at 37°C, but no stress response is induced. However, 42°C is within a degree of the temperature at which these bacteria will cease to grow and begin to die precipitously, and the heat-shock response is induced. The fourth regime was a variable environment with daily alternation between 32°C and 42°C.

Figure 2.5 shows the average rate of genetic adaptation under each regime. For example, the rate of fitness increase for populations that evolved at 32°C was based on competition experiments at 32°C against the common ancestor of all the temperature-selected lines. Genetic adaptation was significantly faster in all of the novel regimes than under the continuation of the ancestral regime (Bennett *et al.* 1992). Environmental change evidently leads to faster genetic adaptation, provided the change is not so extreme as to cause extinction (see below).

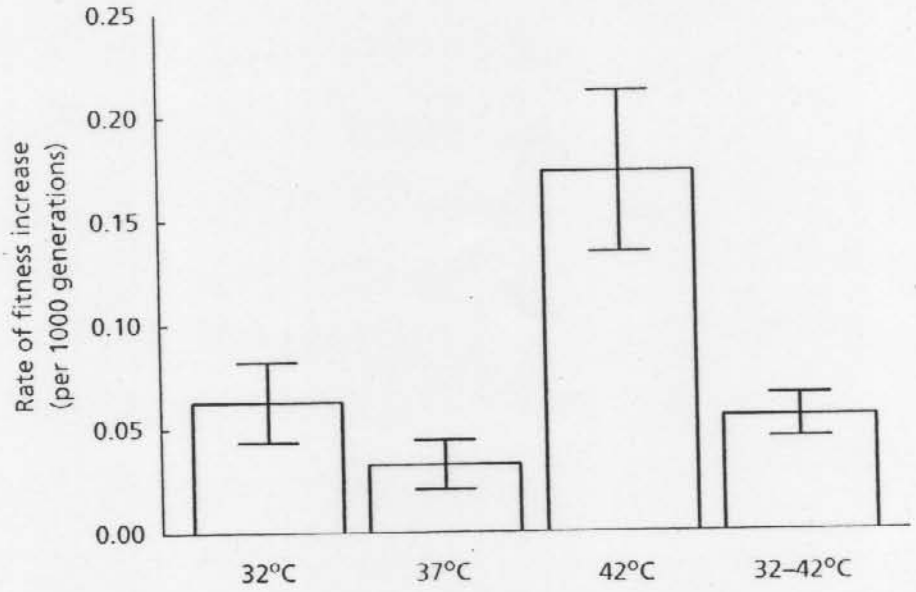

Figure 2.5 Rates of genetic adaptation by *E. coli* to four thermal regimes, measured by improvements in competitive fitness under each regime. For these populations, 37°C is a continuation of the ancestral temperature, 32°C and 42°C are two novel but constant temperatures, and 32–42°C is a novel and variable regime. The height of each bar is the mean of six independent populations; error bars are 95% confidence limits. (Reproduced with permission from Lenski (1995); data from Bennett *et al.* (1992).)

To examine the thermal specificity of genetic adaptation, we measured the correlated fitness responses of the evolved bacteria across a range of temperatures (Fig. 2.6). There was strong specificity in the responses of the three groups that evolved at constant temperatures (Bennett & Lenski 1993). The populations that evolved at 32°C were better competitors than the 37°C-adapted ancestor at 27°C and 32°C, but they were not significantly better at 22°C or 37°C. The populations that evolved at 37°C improved at 37°C, but this improvement did not carry over to 32°C or 40°C. And the populations that evolved at 42°C were better competitors at 40°C, 41°C, and 42°C; but they were not improved at 37°C, nor had they significantly extended their upper limit for growth. By contrast, the populations that evolved in the variable regime, which alternated between 32°C and 42°C, showed the least specificity of adaptation; this group improved in competition at temperatures ranging from 22°C to 42°C.

Given the specificity of genetic adaptation of the groups that evolved under the novel but constant regimes of 32°C and 42°C, one may ask whether they showed trade-offs—or losses of competitive fitness—at the opposite temperature extreme (Bennett & Lenski 1993). Neither group suffered consistent trade-offs, but within both groups, there was heterogeneity among replicate populations in their correlated responses. In particular, the upper growth limit for some 32°C-adapted

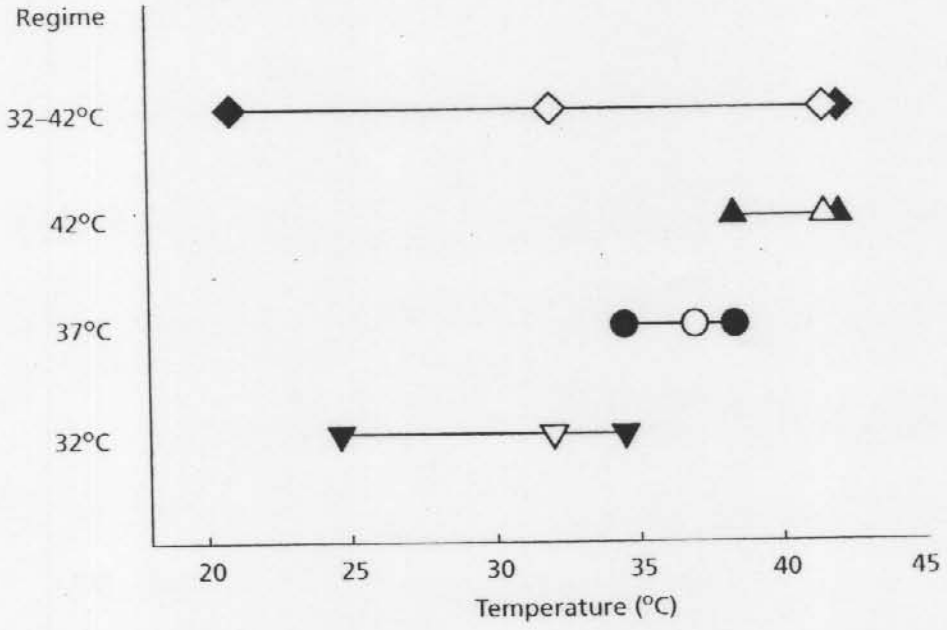

Figure 2.6 The range of temperatures over which *E. coli* populations, adapted to each of four thermal regimes, exhibit improvements in competitive fitness relative to their common ancestor. Open symbols show the temperatures at which populations evolved under each regime. Filled symbols show the approximate range of their fitness improvements. (Reproduced with permission from Lenski (1995); data from Bennett & Lenski (1993).)

populations was shifted down by a degree or so; and one 42°C-adapted population was consistently an inferior competitor at 32°C and below.

None of the changed thermal regimes was so extreme as to cause extinction. That is, all of the regimes were within the ancestor's fundamental thermal niche, defined as the range of temperatures over which the ancestral population could replace itself given the daily dilution into fresh medium (Fig. 2.7). We also studied what happens when the environment changed such that a population found itself above its upper limit for persistence (Mongold *et al.* 1999). These populations declined precipitously by several orders of magnitude, and they usually became extinct. However, a few populations rebounded owing to mutations that extended their fundamental thermal niche. These thermotolerant mutants were consistently inferior competitors within the ancestral niche, even at high temperatures. This trade-off may explain why populations that adapted to 42°C did not show any consistent extension of their upper thermal limit (Bennett & Lenski 1993; Mongold *et al.* 1999). By examining the propensity of populations that previously evolved at different temperatures to give rise to thermotolerant mutants, we sought to test a 'stepping-stone' model, in which populations that evolved at high but non-lethal

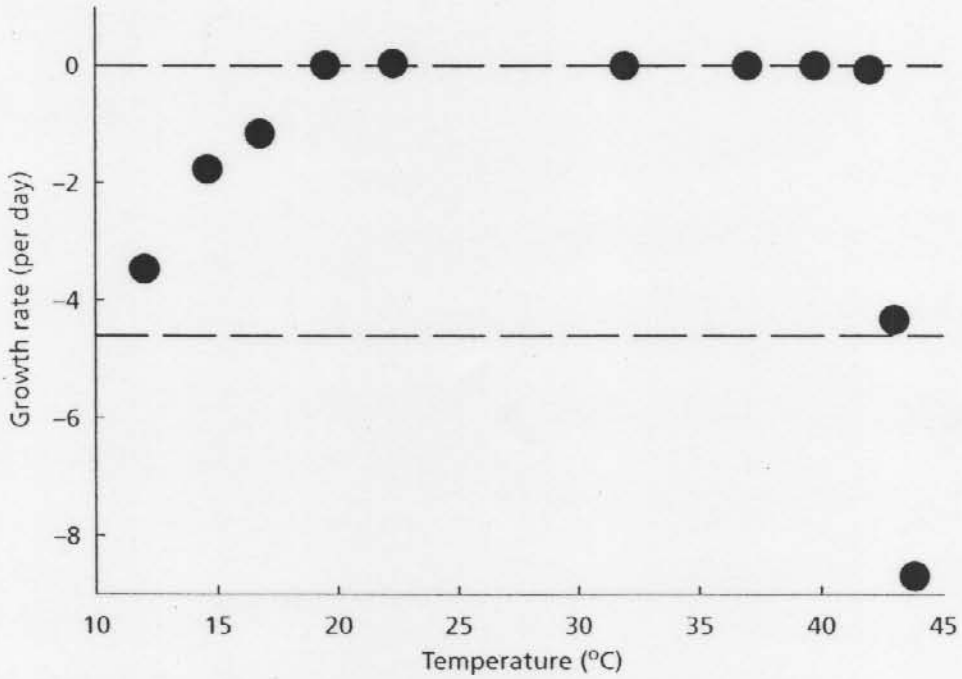

Figure 2.7 The fundamental thermal niche of the ancestral *E. coli* strain used in the experiments on evolutionary adaptation to changing thermal environments. In these experiments, bacteria were diluted 1 : 100 each day into fresh medium. A net growth rate of 0 per day demonstrates population persistence; a net growth rate of –4.6 per day implies dilution without any growth or death. This strain can persist between about 19.5°C and 42°C given these culture conditions. (Reproduced with permission from Bennett & Lenski (1993).)

temperatures may be predisposed to adapt genetically to lethal temperatures (Mongold *et al.* 1999). We observed a tendency in this direction, but the number of cases in which thermotolerant mutants arose was too small to provide compelling support for this hypothesis.

Interactions with viral predators

Bacteria, including *E. coli*, can be infected by viruses (reviewed by Lenski 1988a). In the case of the lytic viruses discussed below, infection is lethal to the bacterium, and the interaction is effectively that of a predator and its prey (or, more precisely, a parasitoid and its host). We have examined the impact of genetic change on the dynamics of interactions between *E. coli* and several viruses in continuous culture (Lenski & Levin 1985; Lenski 1988b). We also studied how evolution influenced the response of these communities to changes in environmental productivity (Bohannan & Lenski 1997, 1999, 2000).

Figure 2.8 shows the dynamics of *E. coli* and virus T4 in a chemostat during about 500 h. Initially, the viruses held the bacterial population in check at a density far below its resource-limited equilibrium density. Soon, however, T4-resistant

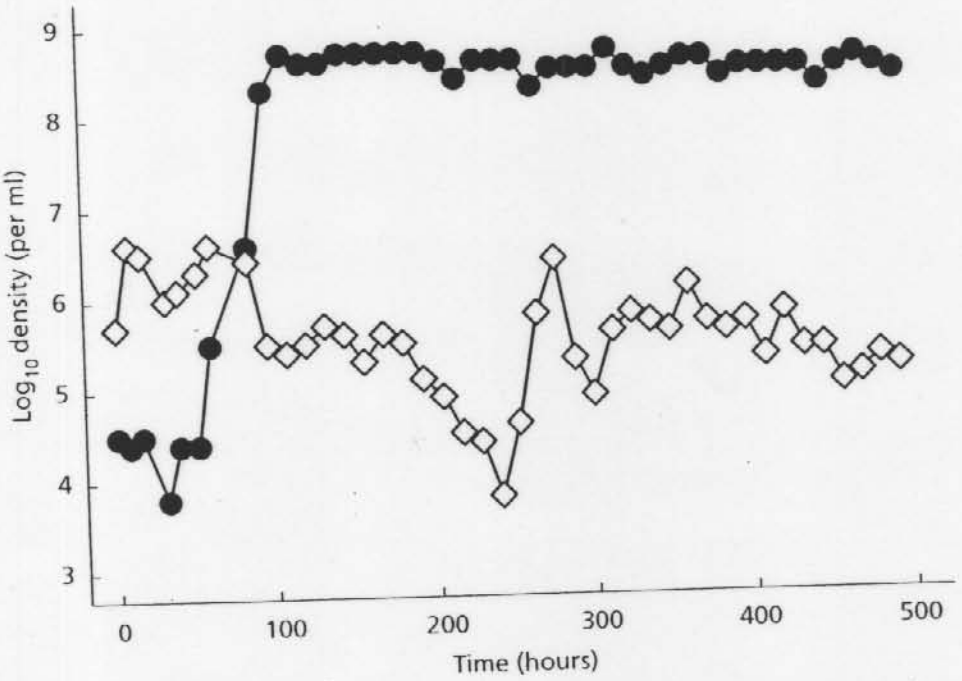

Figure 2.8 Dynamics of interaction between populations of *E. coli* (filled circles) and virus T4 (hollow diamonds) in chemostat culture. The rapid and large increase in the *E. coli* population after about 60 h reflects the emergence of a mutant that is completely resistant to T4 infection. The T4 population persists after the emergence of resistance on a minority population of sensitive cells, which themselves persist owing to their superior competitive ability for glucose. (Reproduced with permission from Lenski & Levin (1985).)

bacterial mutants appeared, and they grew quickly until they reached their resource-limited equilibrium; at that point, the glucose concentration was at a level where the growth of the resistant bacteria matched the rate at which they were washed out of the culture vessel. These mutants were completely resistant, and they did not revert to a sensitive form at any measurable rate (Lenski 1988c). Furthermore, unlike some viruses, T4 did not generate 'host-range' mutants that were able to infect the resistant mutants (Lenski & Levin 1985). Thus, when viruses were introduced to a pure culture of resistant bacteria, the viruses became extinct (Lenski & Levin 1985). However, the virus population did not become extinct following the evolution of resistant mutants (Fig. 2.8).

The T4 population persisted because the resistant bacteria were inferior competitors for glucose (Fig. 2.9), as was shown by mixing resistant and sensitive genotypes in the absence of the virus (Lenski & Levin 1985; Lenski 1988b). Therefore, when resistant mutants increased to their resource-limited equilibrium in the presence of viruses, the amount of glucose was still sufficient to support growth of the sensitive bacteria above the level needed to offset their losses by washout. This excess growth of the sensitive bacteria was exploited by the virus population, which persisted while holding in check the superior competitor. In effect, the community evolved from a two-member food chain to a three-member structure in which a

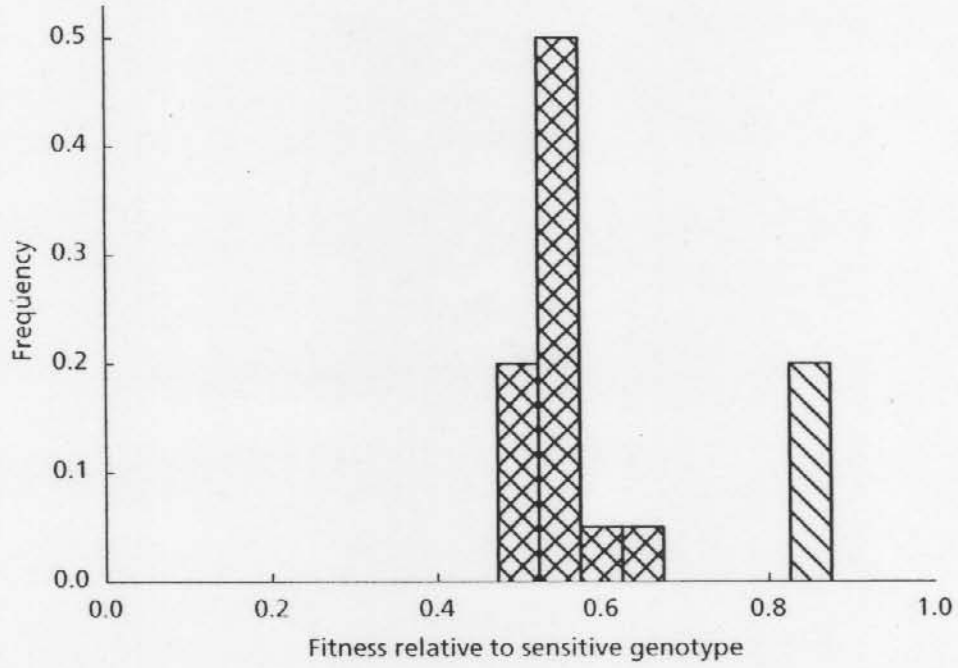

Figure 2.9 Distribution of the competitive fitnesses of 20 mutants of *E. coli*, each resistant to virus T4 infection, relative to their sensitive progenitor. Competitions were performed in glucose without virus. Hatched and cross-hatched fills indicate mutants that were sensitive and cross-resistant to another virus, T7. (Reproduced with permission from Lenski (1988b).)

keystone predator mediated the coexistence of two genotypes at the lower level by preventing competitive exclusion.

Evolution of the interaction increased diversity, and it also fundamentally altered the community's response to environmental change (Bohannan & Lenski 1997, 1999). We manipulated environmental productivity by varying the inflow of glucose to the community. Table 2.1 summarizes the responses to increased productivity of the two-member food chain and the three-member community with virus-mediated coexistence of the sensitive and resistant genotypes. In the simple food chain, the equilibrium density of the T4 predator increased substantially at higher productivity, whereas the bacteria increased only slightly. Moreover, the community was less stable at higher productivity, with increased fluctuations in both populations (Bohannan & Lenski 1997). By contrast, in the three-member community, the total density of the bacteria, but not of the virus, increased at higher productivity. Within the bacteria, only the resistant genotype increased in abundance, essentially drawing down the extra resource such that none of it was available to support faster growth of the sensitive genotype and more viruses. These effects agree well with mathematical models of two- and three-member communities (Leibold 1996), and they show that fundamental changes in both a community's structure and its dynamical properties can arise quickly as a consequence of genetic change.

The trade-off in the bacteria between their competitive ability and resistance to viral infection is an important feature of this interaction. We also studied interactions between *E. coli* and three other lytic viruses (Lenski & Levin 1985). Against virus T5 only, the bacteria suffered no cost of resistance. As a consequence, the sensitive bacteria, and with them the viruses, were driven to extinction following the evolution of resistance. Even in the case of virus T4, the trade-off between bacterial

Table 2.1 Impact of the emergence of an *E. coli* mutant resistant to virus T4 on the community's responses to changes in environmental productivity. The two-member community contains sensitive bacteria and the viral predator; the three-member community includes both sensitive and resistant genotypes and the virus. Productivity was manipulated by varying glucose influx into replicate communities. (Summary of data from Bohannan & Lenski (1997, 1999).)

Effect of increased environmental productivity on:	Two-member community	Three-member community
Density of bacteria	↑	↑↑
Density of sensitive genotype only	NA	↔
Density of resistant genotype only	NA	↑↑
Density of viruses	↑↑	↔

↔, no significant change; ↑, small increase; ↑↑, large increase; NA, distinction between bacterial genotypes is not applicable.

resistance and competitive ability was complex (Lenski 1988b,c). In the absence of T4, one might expect the bacteria would revert to sensitivity. However, evolutionary reversions rarely occurred, probably because mutations to T4 resistance usually knocked out the genes that encode the surface receptor used for viral attachment. Instead, evolving populations of resistant bacteria underwent compensatory changes that significantly reduced the cost of resistance (Lenski 1988c).

Another important feature of the interaction between *E. coli* and virus T4 is that resistance was complete. However, in the case of interactions between *E. coli* and virus T2, the initial mutations conferred only partial resistance (Lenski 1984; Bohannan & Lenski 2000). The ecological consequences of partial resistance are very different from those of complete resistance. With complete resistance, and given the trade-off with competitive ability, the resistant and sensitive genotypes coexisted in the presence of the viral predator. However, partial resistance to virus T2 led to a situation of 'apparent competition', which refers to the indirect interaction between two prey populations that share a predator (Holt 1977; Holt *et al.* 1994). The density of the partially resistant bacteria remained under the control of the virus, and when the environment was sufficiently productive the emergence of the partially resistant genotype caused an increase in the abundance of viruses, more of which were required to hold this prey population in check. The higher density of T2 increased the level of predation on the sensitive bacteria beyond what they could sustain, and they were driven to extinction as a consequence of this feedback involving the shared predator (Fig. 2.10). In less productive environments, by contrast, the sensitive bacteria prevailed owing to their superiority in competition for glucose (Fig. 2.10). Thus, the resulting community structure, and the effect of environmental productivity thereon, depended on whether mutations gave rise to bacteria with partial or complete resistance to the virus.

Interpretation and synthesis

All of our experiments demonstrate 'a genetic component' to 'the abundance and distribution of organisms'—although it was probably a bit *too* strong to call this 'basically a genetic problem' (Tenet 1). The genetic component to organism abundance is dramatically illustrated by the fact that a single mutation, one which conferred resistance to infection by virus T4, changed the density of a bacterial population by several orders of magnitude (see Fig. 2.8). This genetic factor must be placed on an equal footing with the environmental factor that would otherwise control the bacterial population; that is, the density of bacteria following the evolution of resistance was very nearly the same as the sensitive population's density in the absence of any virus. In the case of the interaction between *E. coli* and T5, the resistance mutation was even more important: in addition to the increase in the bacteria to their resource-limited density, the virus population became extinct because the sensitive bacteria had no advantage in competition for glucose. In the case of virus T2, the effect of partial resistance on bacterial abundance was more subtle; partially resistant bacteria sometimes displaced their fully sensitive

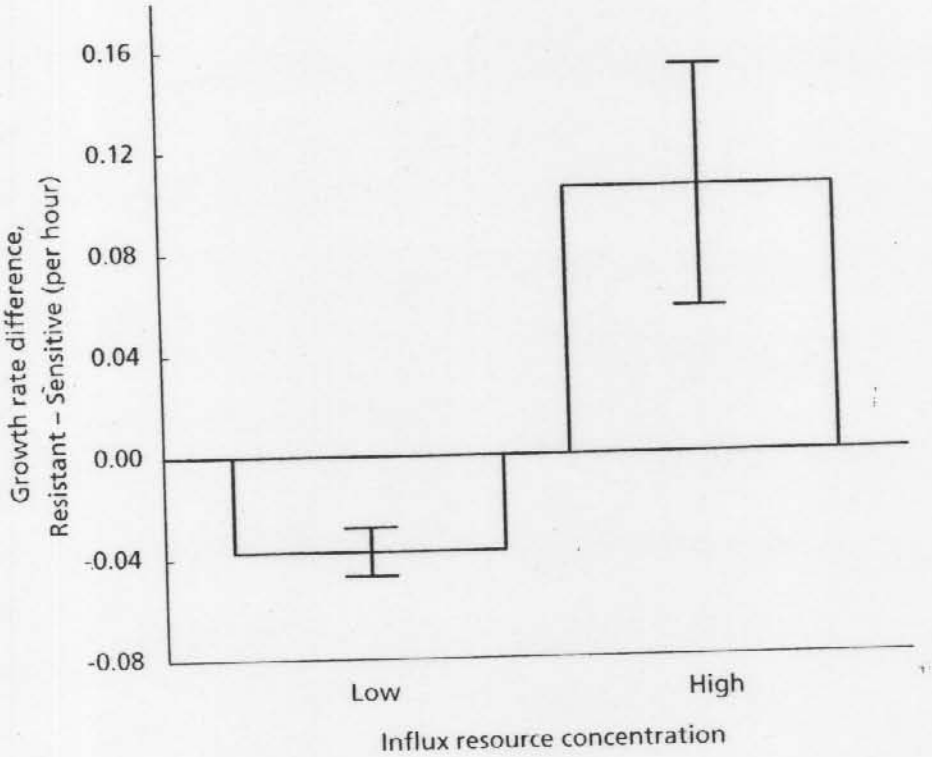

Figure 2.10 Competition between an *E. coli* mutant that is partially resistant to virus T2 and its sensitive progenitor, performed in the presence of T2 at both low and high glucose influxes. The partially resistant genotype prevails at high influx concentration (0.5 μg/mL), whereas the sensitive genotype dominates at low influx concentrations (0.07–0.12 μg/mL). Error bars are 95% confidence intervals. (Data from Bohannan & Lenski 2000.)

progenitors, but the bacterial population remained predator limited (Fig. 2.10). Genetic resistance by plants and animals to consumers (herbivores as well as pathogens and parasites) also may be either partial or complete, depending on the specific mechanism of resistance. As shown by our experiments with bacteria and viruses as well as by mathematical models, this distinction can have profound consequences for the ecological amplitude of the interacting species and the structure of the community in which the interaction is embedded.

Even in our single-species experiments, the abundance of *E. coli* changed as they adapted genetically to growth on glucose. The populations experienced a counterintuitive decline in numbers, but an increase in overall biomass as the average individual bacterium became much larger (see Fig. 2.2). Also, the range of environmental temperatures over which the bacteria persisted—in effect, their distribution—had a genetic component, as occasional mutations arose that allowed bacteria to reproduce at temperatures that were lethal to their progenitors.

The studies of evolving bacteria support the view that 'forces maintaining species diversity and genetic diversity are similar', and they show that 'an understanding of community structure [can] come from considering how these kinds of

diversity interact' (Tenet 2). One example is the stable coexistence of two bacterial genotypes on glucose (see Fig. 2.4). As would any stable coexistence, it depended on a trade-off between certain ecological capacities, in this case exploiting glucose and scavenging metabolites. In effect, the genotypes not only partitioned the resources, but through their own activities they produced the resource complexity that allowed them to coexist (Rosenzweig *et al.* 1994). Other striking examples of the basic similarity between forces maintaining species diversity within communities and genetic diversity within populations come from the interactions between bacteria and viruses (see Fig. 2.8). The coexistence of T4-sensitive and -resistant genotypes mirrors the concept of a keystone predator that maintains species diversity by disproportionately preying on the most competitive species (Paine 1966; Lubchenco 1978). The extinction of virus T5 after resistance evolved emphasizes the importance to coexistence, whether of species or genotypes, of the trade-off between competitive ability and resistance. The dynamical tension between scramble competition for resources and apparent competition mediated by a predator (Holt 1977; Holt *et al.* 1994) is seen in the dependence on environmental productivity of the fate of mutants partially resistant to virus T2.

All these parallels between the forces that maintain genetic diversity and species diversity reflect interactions that are density dependent. Of course, there are other forces that can maintain genetic diversity within populations, including heterozygote superiority and mutation–selection balance, that have no direct counterparts with respect to maintaining species diversity.

Another interesting aspect of this tenet is understanding how genetic and species diversity interact to influence community structure. The experiments with bacteria and virus T4 illustrate the very strong interaction between these levels of diversity. In the absence of T4, resistant bacteria were rare and inconsequential. In the presence of virus, the resistant genotype not only became numerically dominant but also altered the community's structure and responsiveness to certain kinds of environmental change (see Table 2.1). Prior to the evolution of resistance, the bacterial population was predator limited while the virus population was limited by excess growth of the bacteria, which depended on the productivity of the environment. Thus, in response to increased resources, the growth rate of the bacteria increased, which led to a much higher density of viruses, a slight increase in the bacterial population, and a destabilization of both populations. After the resistant genotype evolved, these mutants drew down the free resource, which reduced the growth rate of sensitive bacteria and thus lowered the equilibrium density of the virus while also stabilizing the interaction. Moreover, the productivity response of the community with genetically diverse prey population was essentially inverted, such that the bacteria but not the viruses increased in density as more resources became available. These experiments show that a community's performance, as well as its structure, can be fundamentally altered by genetic diversity within its members.

All three sets of experiments also show clearly that 'adaptation is a dynamic process' and that 'Darwinian fitness can be measured' as the differential reproductive success of genetically distinct individuals within populations (Tenet 3).

Whether the bacteria adapted to glucose (see Fig. 2.1), to changes in temperature (see Fig. 2.5), or to viral predators (see Fig. 2.8) depended of course on the environment, as did their rate of adaptation. But in every case the bacteria became better adapted to those conditions than were their ancestors, as demonstrated by allowing different genotypes to compete under the relevant regimes. We also tested whether adaptation to one environment led to maladaptation in other environments, which may indicate genetic trade-offs between ecological functions (see Figs 2.3, 2.6, 2.9 and 2.10).

Of course, it is one thing to measure genetic adaptation in simple laboratory systems; it is obviously more difficult to do so in the natural communities that most ecologists study. However, considerable advances have recently been made in studying adaptation even under the most difficult circumstances. These advances are reflected in a long-term study of Darwin's finches (Grant 1999), and more generally by a growing body of work that seeks to develop and apply new methods for studying adaptation (Lande & Arnold 1983; Endler 1986; Harvey & Pagel 1991; Reznick *et al.* 1997; Thompson 1998, 1999; Clutton-Brock *et al.* 1999; Huey *et al.* 2000; Rundle *et al.* 2000). Regardless of any difficulty, genetic adaptation must be demonstrated before we can move from mere speculation to hypothesis testing about its role in the structure and function of ecological communities. Moreover, by investigating the actual dynamics of genetic adaptation, one obtains information about the time course over which evolution can exert a significant influence on ecological communities. It will certainly be interesting to better understand how the pace of evolutionary change compares with other processes that impact communities, such as climate change, habitat destruction and species introductions.

These experiments also demonstrate that 'adaptation to new environments will result in different genotypes with different life histories', and that 'genetic adjustment . . . may occur by direct response . . . or by compensatory change' (Tenet 4). The bacteria genetically adapted to the limiting resource, temperature regime and to the presence of viral predators. Certain genetic adaptations, but not others, led to trade-offs in different environments. For example, mutants that were resistant to virus T4 were less competitive for glucose, whereas mutants resistant to virus T5 did not incur this cost. Similarly, some populations adapted to high temperature lost fitness at low temperatures, whereas others did not.

Predicting when trade-offs will occur is difficult owing to the inherent genetic and physiological complexity of even simple organisms. Trade-offs may often play a major role in maintaining diversity, but they cannot automatically be assumed to be important and must be experimentally tested (Simms & Rausher 1989; Biere & Antonovics 1996; Kraaijeveld & Godfray 1997). Moreover, even when genetic trade-offs do arise, they are not necessarily immutable constraints on adaptation. Instead, some trade-offs can be ameliorated by compensatory changes that modify the deleterious side-effect of the selected trait without diminishing that trait itself (McKenzie *et al.* 1982). In our work with bacteria, we have seen compensation for the costs of resistance to both viruses (Lenski 1988c) and antibiotics (not discussed

here, but see Bouma & Lenski 1988; Lenski *et al.* 1994), which, in the latter case, has troubling implications for public health (Lenski 1997). Antonovics' attention to compensatory as well as direct change reminds us that the fate of any mutation depends not only on its ecological context but also the genetic 'environment' in which it occurs.

Perhaps Antonovics' most provocative tenet is that 'the distinction between "ecological time" and "evolutionary time" is artificial and misleading', and that 'frequently genetic and ecological changes are simultaneous' (Tenet 5). In the long-term study of bacteria living on glucose, the two time scales seem fairly distinct. Genetic changes in competitiveness, morphology and physiology evolved over hundreds and thousands of generations, but these changes did not prevent us from measuring relative fitness by allowing different strains to compete for several generations (see Figs 2.1–2.3). In fact, evolution was sufficiently slow that we could observe the ecological dynamics leading to the stable coexistence of two ecotypes over a period of a hundred generations or so (see Fig. 2.4). However, when we examined these ecotypes over a much shorter time scale—the seven generations spanning the different 'seasons' (growth phases) within each transfer cycle—we saw that their relative abundance changed depending on the availability of glucose and secondary metabolites in the medium. And over thousands of generations, the identity of the ecotype that was numerically dominant changed repeatedly, a dynamic which may reflect their continuing genetic adaptation to one another or their common environment. Thus, even in this simplest of our experimental systems, one cannot draw any absolutely consistent distinction between ecological and evolutionary scales. More generally, changes in genotype frequencies (at least those that occur by natural selection acting at the level of individuals) necessarily imply differences in demographic parameters. Therefore, it must be true at some level that genetic adaptation has a simultaneous ecological manifestation, even if it is very subtle.

The experiments under different thermal regimes show more obvious convergence in ecological and evolutionary time scales. Genetic adaptation was faster in each of the novel environments than in the continuation of the ancestral environment, indicating that ecological change promotes evolution (see Fig. 2.5). The emergence of mutants that maintained populations at temperatures lethal to their progenitors provides an example in which ecological and genetic changes were essentially simultaneous. Moreover, the ecological fate of the population—extinction or persistence—hinged on a genetic event, illustrating the feedback between ecological and genetic changes.

The most compelling example of the correspondence between ecological and evolutionary time comes from our experiments with bacteria and viruses. Within a matter of a few days, bacterial mutants resistant to viral infection emerged and radically altered the population and community dynamics (see Fig. 2.8 and Table 2.1). By the time the interacting virus and sensitive bacteria went through a few predator–prey cycles, these dynamics were substantially changed by the appearance of resistant mutants (Bohannan & Lenski 1997). In fact, the extreme rapidity

of this genetic change made it difficult to study the 'purely ecological' dynamics of this system.

These three sets of experiments with bacteria together illustrate the range of possibilities with respect to distinguishing ecological and evolutionary time scales. On the one hand, in simple and constant environments, genetic change may be sufficiently slow that it does not profoundly undermine one's ability to make ecological inferences. On the other hand, in more complex and novel environments significant genetic change becomes more likely and rapid, and these evolutionary responses will in turn affect the ecological performance of populations and communities. The faster pace of change in more complex and novel environments presumably derives from the fact that organisms living in such environments are generally further from a peak in the fitness landscape than those organisms that live in simpler, more stable environments. The fact that organisms living under more difficult circumstances should often have smaller population sizes may partially offset the tendency for more rapid genetic adaptation in complex and novel environments, especially in extreme cases. For example, only a small proportion of bacterial populations that were challenged with a lethally high temperature produced a thermotolerant mutation before becoming extinct (although those few populations that did survive underwent very rapid change).

A sceptic might say that ecologically significant genetic changes are more likely in bacteria than in plants and animals, owing to the faster generations and larger population sizes of bacteria. However, this view confuses what one can readily measure with what is actually important. Evolutionary changes are, of course, more easily observed in microbes than in plants and animals. But if studies of plants and animals also lasted hundreds and thousands of generations, and given the vast size of many natural populations, who would doubt the potential for widespread genetic change in ecologically important traits? Moreover, there are numerous examples with plants and animals in which ecologically significant genetic changes have occurred within only a few generations, with some of the most conspicuous cases involving interspecific interactions and resistance to chemical agents (McKenzie 1996; Thompson 1998).

Concluding remarks

Life on earth has existed for billions of years, and we can hope that it will continue long into the future. But now and for the foreseeable future, many ecosystems will experience extremely rapid environmental change owing to human activities, including climate change, habitat destruction and the introduction of non-native species (Wilson 1988; Kareiva *et al.* 1993; Simberloff *et al.* 1997). It is precisely such changing circumstances that promote rapid evolution, with its attendant consequences for populations and communities. The on-going extinction of the earth's biota may be similar to major extinctions in the geological past (Raup 1988; Vermeij 1991). Patterns from the fossil record, including time-lagged concordance between rates of extinction and origination of new taxa, indicate that evolutionary

change in surviving lineages and communities was often concentrated in and after tumultuous periods (Vermeij 1991; Jablonski & Sepkoski 1996; Erwin 1998; Jackson & Cheetham 1999; Weil & Kirchner 2000). Thus, in our present period of extremely rapid environmental change it becomes especially important that we integrate ecological and genetic studies in an evolutionary framework.

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