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THE ECOLOGY AND GENETICS OF MICROBIAL DIVERSITY

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■ **Abstract** Natural communities of microbes are often diverse, a fact that is difficult to reconcile with the action of natural selection in simple, uniform environments. We suggest that this apparent paradox may be resolved by considering the origin and fate of diversity in an explicitly ecological context. Here, we review insights into the ecological and genetic causes of diversity that stem from experiments with microbial populations evolving in the defined conditions of the laboratory environment. These studies highlight the importance of environmental structure in governing the fate of diversity and shed light on the genetic mechanisms generating diversity. We conclude by emphasizing the importance of placing detailed molecular-level studies within the context of a sound ecological and evolutionary framework.

CONTENTS

THE PROBLEM OF DIVERSITY AND THE NICHE EXCLUSION PRINCIPLE

Most natural communities are highly diverse, and microbial communities appear to be no exception (28, 38, 70, 100, 107, 126, 134). Application of molecular techniques to the study of microbial diversity (98, 101) has revealed the existence of an incredible variety of genotypes and species in all known habitats—many of these types appear to be ecologically equivalent in terms of resource requirements (107, 112). For example, recent theoretical (28) and empirical (125) analyses of the microbial diversity of soils indicate that soils harbor in the order of 7000 different taxa at an abundance of approximately 10^9 cells per cubic centimeter. The existence of such diversity demands explanation because it is at odds with the theoretical expectation that natural selection eliminates all but the best-adapted type under any given set of environmental conditions. How, then, can such high levels of diversity be maintained in natural microbial communities?

Conventional approaches to microbiology have not supplied a satisfactory answer. Indeed, microbial ecologists have tended to sidestep the problem, focusing attention almost exclusively on the documentation of diversity. Ecologists and population geneticists, on the other hand, have long grappled with issues relating to the maintenance of diversity: Much of the motivation for research in ecology, population genetics, and evolution has been driven by the need to explain the paradox of diversity (61, 86, 123). Despite different approaches (ecologists being concerned with the diversity of species in communities and population geneticists with the variety of genotypes within populations), both ecologists and population geneticists have identified similar principles governing the fate of diversity in natural environments.

Paramount among these is the niche exclusion principle (47, 54), which states that a single niche can support no more than one type, whether it be a genotype or a species. In this review, a niche is the variety of factors in an environment that limit the growth of one type relative to others. A limiting factor may be an essential resource or nutrient; a set of physical conditions such as temperature, pH, or salinity; or the existence of refuges from predation. According to this view, the environment can be interpreted as the number of niches available to a lineage or lineages, and the number of types supported in an environment determined by the number of niches (85, 122). As will become apparent, this statement requires further qualification but nevertheless forms a useful starting point. Note also that the concept of niche has a long and tortuous history, and we refer readers to books by Elton and Whittaker, and Chase & Leibold (42, 132, 135) for a full account.

In this article we highlight studies that have investigated the emergence or fate (or both) of microbial diversity. We draw primarily upon investigations that use simple experimental populations of microbes propagated in highly controlled environments where the structure of the environment—i.e., the number of niches and the manner in which they vary in space and time—is determined by the experimenter. We focus on these studies because, by virtue of their simplicity, they provide insight into the mechanistic causes of diversity and its maintenance. Such insights are difficult (if not impossible) to obtain from the analysis of complex natural communities. Our concern here is with the origin and fate of genetic variance in fitness (see below). We do not consider the functional significance of this diversity, which, although important, is beyond the scope of this work. Interested readers may consult Loreau et al. (88) for entrance into this literature.

THE REVEREND DALLINGER AND MECHANISM IN MICROBIOLOGY

For most microbiologists, "mechanism" refers to the biochemical and physiological interactions occurring among genes and their products in a cell. To understand diversity in populations and communities, it is necessary to broaden this perspective to incorporate fitness. Fitness, a measure of the ability of one genotype to leave offspring relative to other types, is the ultimate arbiter of ecological success. The mechanisms underlying changes in fitness are the interactions among genotypes and between genotypes and their environment.

To illustrate this, consider an experiment reported by the Reverend William Henry Dallinger, a Wesleyan clergyman and president of the Royal Microscopical Society, over a century ago. In his president's address to the Society for 1887, Dallinger asked "whether it was possible by change of environment, in minute life-forms, whose life-cycle was relatively soon completed, to superinduce changes of an adaptive character, if the observations extended over a sufficiently long period" (30, p. 191). He started with a collection of microorganisms (what he termed monads) and grew them in a purpose-built incubator, gradually increasing the temperature over seven years until the apparatus was accidentally destroyed. Just before the accident Dallinger returned his experimental populations, which by now were growing at temperatures well beyond their normal thermal tolerance limits, to the temperature at which he had started the experiment. None grew. He reasoned that the lines had adapted to the increasing temperatures by natural selection and in so doing had lost the ability to grow at their normal temperature.

Note that mechanisms invoked to explain the results of this experiment are fairly simple: Natural selection caused by increasing temperatures leads to adaptation, and this comes at the cost of not being able to survive at the ancestral temperature. No knowledge of the molecular genetics, biochemistry, or physiological causes of adaptation is required to interpret these results, although such knowledge is highly desirable. The fact that the derived populations could no longer grow in

the ancestral environment demonstrates that the changes that occurred during the course of the selection experiment were genetically determined. Had Dallinger's experiment not been destroyed, he might well have proceeded to determine the magnitude of improvement at elevated temperatures relative to the ancestral type, tested for and directly measured tradeoffs in ecological performance at different temperatures, and even documented diversity within evolving populations (its stability and changes in patterns of abundance through the course of the experiment). The remarkable fact is that with relatively little additional development, the approach Dallinger took more than a century ago remains a valid and powerful means of studying some of the most fundamental problems in biology. Moreover, such analyses can provide insight into the mechanism of ecological and evolutionary change—with the same kind of power and precision that a molecular microbiologist expects when tackling, for example, a problem in gene regulation.

The experiment also highlights a second issue of importance for microbiologists interested in understanding the causes of adaptation and diversity, namely that evolution can be studied on timescales that are amenable to experimentation and analysis. This should come as no surprise, as the rapid generation times and large population sizes of microbial populations are ideal conditions for natural selection. Interestingly, Darwin himself missed the opportunity to test experimentally his theory of adaptation by natural selection: In a letter to Dallinger dated July 2, 1878, Charles Darwin wrote: "I did not know that you were attending to the mutation of lower organisms under changed conditions of life; and your results, I have no doubt, will be extremely curious and valuable. The fact which you mention about their being adapted to certain temperatures, but becoming gradually accustomed to much higher ones, is very remarkable. It explains the existence of algae in hot springs. How extremely interesting an examination under high powers on the spot, of the mud of such springs would be" (30, pp. 191–192).

TECHNIQUES AND METHODS

The techniques for studying adaptation and diversity in the laboratory have not changed much from Dallinger's time. They are conceptually straightforward and borrowed directly from quantitative genetics, agronomy, and animal breeding (4, 37, 41, 43, 64, 79, 104).

Briefly, a population is allowed to grow and reproduce in an environment that is defined by the experimenter. Most commonly, these environments are batch cultures of simple media (82, 90, 95), although continuous cultures (33, 97, 110) and plate cultures (65, 71, 76) are sometimes used. The duration of the experiment depends on the theory being tested. For ecological experiments, it is common to start with a highly diverse population (or collection of species) and follow the fate of diversity over the course of a few tens of generations (94, 116). Evolutionary experiments typically begin with a single strain and last for hundreds or thousands of generations, new variation arising during the course of the experiment through

de novo mutations. Variation that arises through mutation or pre-exists in the population generates competition among genotypes as the population grows and uses up the available resources. The type that is fittest, in the sense of being the best competitor under the prevailing conditions of growth, comes to dominate the population through natural selection. This leads to a change in the genetic makeup of the population and the steady replacement of one genotype by another. The evolved populations therefore out-compete the ancestor in the environment in which they have been selected.

Fitness itself can be measured in either of two ways. The first estimates growth parameters such as *r*, the intrinsic rate of growth, and *K*, the carrying capacity, in pure culture. The second involves competing each genotype against a common ancestor and measuring the rate of competitive exclusion (37, 82). The latter is preferable because it is an integrated measure that takes into account adaptation to the abiotic and biotic conditions of growth. To estimate the quantity of diversity in a population, it is necessary to isolate individual genotypes from the population and estimate the variance in fitness among them.

THE STRUCTURE OF THE ENVIRONMENT AND THE MAINTENANCE OF DIVERSITY

Ecologists and population geneticists have long-suspected that the structure of the environment is connected to the maintenance of diversity (64). It is easy to see why this might be so. Natural selection should eliminate all but the fittest type under any given set of growth conditions, leading to a loss of diversity. Reasonably, then, if the environment were not uniform but instead a series of distinct niches (or patches) that differed in their conditions of growth, then different types may be favored in each niche, and so diversity maintained according to the niche exclusion principle.

The variety of available niches, and therefore the variety of types supported, may be determined by the physical structure of the environment, for example, by the patchy distribution of essential resources. The variation in fitness of genotypes in response to the abiotic environment is known in population genetics as genotype-by-environment interaction, or GxE (66, 129). Alternatively, new niches may arise through the actions of organisms themselves. In such instances diversity is maintained through genotype-by-genotype interactions, or GxG (4). As we shall see, experimental studies with microbial populations in the laboratory provide support for both kinds of interactions.

Simple Environments Composed of a Single Niche

Imagine a single genotype of an asexual microorganism inoculated into a simple environment composed of a single niche to which it is initially not well adapted. As the population grows, mutations arise randomly with respect to fitness and lead to

genetic diversification. While the vast majority of new mutations are deleterious, a small fraction will be beneficial, in the sense that they confer a higher rate of replication. This growth rate advantage may manifest in different ways. For example, some variants may have a shorter lag time, higher intrinsic growth rates owing to more efficient metabolism, or higher yields for a given unit of resource. Regardless, some of these more fit genotypes will come to dominate the population (for a discussion of constraints on the fixation of beneficial mutations in asexual populations, see References 32 and 48). This is the process of periodic selection, first documented more than half a century ago (2, 3, 97), whereby beneficial mutations are substituted through the population, supplying a transient source of genetic variation that is eventually exhausted as the population nears evolutionary equilibrium.

It is no trivial matter to study periodic selection in microbial populations, as it requires identifying beneficial mutants when they first arise at low frequency in a population and following their fate through time. Atwood et al. (2) achieved this by monitoring fluctuations in the frequencies of his+ prototrophs relative to numerically dominant his- auxotrophs in evolving *Escherichia coli* populations. The authors observed a saw-tooth trajectory of increases and sudden decreases in the frequency of the minor his+ type, and reasoned that the increases were due to recurrent mutation and the decreases were caused by the purging effect associated with the "sweeping" of successive beneficial mutations that arose in the numerically dominant his- population.

Direct support for the operation of periodic selection and concomitant diversity-purging effects come from two recent molecular-level analyses. The first, by Notley-McRobb & Ferenci (96), repeats and extends the classic work of Atwood et al. by tracking the frequency of T5-resistant cells (the equivalent of the his+ marked population used by Atwood et al.) during adaptation to a glucose-limited chemostat. They observed the expected saw-tooth pattern and also showed that decreases in the number of T5-resistant cells corresponded exactly to the sweep of beneficial mutants (Figure 1). Interestingly, they also noted the maintenance of diverse alleles at the selected locus following a sweep, suggesting the existence of different genetic routes conferring similar increases in fitness in these populations.

Going a step further, Wichman et al. (133) sequenced multiple genomes of the DNA bacteriophage ϕX174 after adaptation to a novel host at high temperatures. This allowed the identification of genetic changes and provided a means of tracing the frequency of each change during the course of the experiment. Wichman et al. detected more than a dozen nucleotide substitutions and found strong evidence, on the basis of parallel evolution of the same nucleotide changes and the rapid substitution of unique changes, that most of these substitutions were adaptive. The pattern of substitution indicated a series of rapid sweeps occurring one after the other in a manner consistent with the model of periodic selection described above (Figure 2). Of particular note is that in each population at the end of the experiment, just a single genotype was present. Taken together, these experiments are entirely consistent with the niche exclusion principle and suggest that,

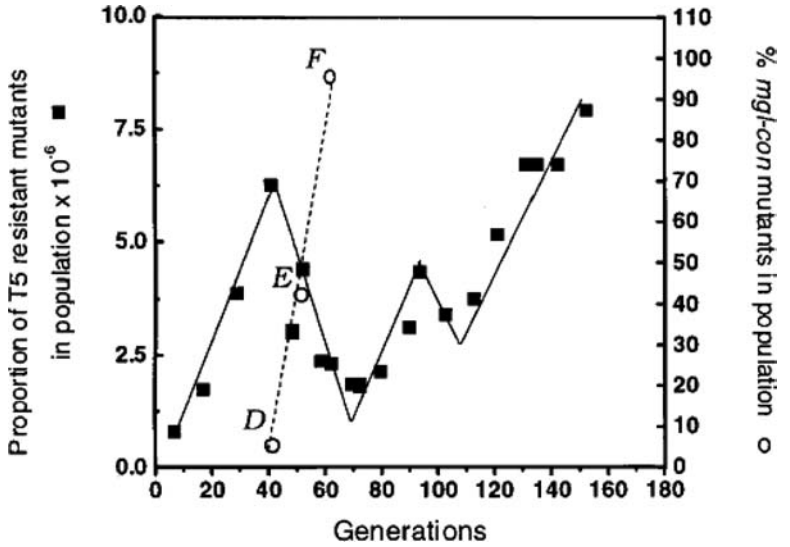

Figure 1 Periodic selection in populations of *E. coli* evolving in a simple, uniform environment. Note the saw-tooth pattern (*solid line*) in the frequency of the rare T5-resistant mutant. Decreases in T5 resistance are correlative with the selective sweep of the beneficial *mgl-con* mutant (*dashed line*). Reproduced with permission from Notley-McRobb and Ferenci (96).

as a general rule, simple, uniform environments cannot maintain genetic variation in fitness in the long term (104).

In apparent contradiction to the niche exclusion principle, however, a number of studies have detected substantial phenotypic and molecular variation in populations evolving in what should be single-niche environments. For example, DNA fingerprinting of insertion sequence (IS) elements in two populations of *E. coli* propagated for over 10,000 generations in glucose-limited minimal medium (79) revealed that 11 of 11 clones in one population and 10 of 13 clones in another had unique IS fingerprints (102). Clearly, diversity existed in these populations, but whether this molecular variation reflected ecologically relevant genetic variation in fitness needed to be determined.

To address this issue, Elena & Lenski (40) estimated the genetic variation in fitness for six replicate *E. coli* populations at generation 10,000 by competing 25 independently isolated clones from each population against their common ancestor. Statistically significant genetic variation in fitness was detected, with two random clones from the same population differing on average by ∼4%. Although this difference was relatively minor in comparison to the roughly 50% increase in fitness relative to the ancestor since the start of the selection experiment (it also varied among replicate populations), it was higher than would be expected through the operation of periodic selection alone in a single-niche environment.

At least three mechanisms could potentially support such low but significant levels of genetic variation in fitness in these populations. First, the populations may have still been adapting to the conditions of growth, albeit at a reduced rate relative to the start of the experiment. Any genetic variation present in the population may, then, reflect the ongoing substitution of beneficial mutations, as in the ϕX174 experiment above. None of the populations, however, showed evidence for increases in mean fitness between generation 10,000 and generation 10,500, as would be expected if beneficial mutations were being substituted. Second, the populations may have reached an evolutionary equilibrium with the majority of new mutations being deleterious and kept at low frequency by natural selection (52). The fortuitous evolution of two "mutator" populations with high mutation rates caused by defective methyl-directed mismatch repair (115) permitted this idea to be tested directly. The prediction that the mutator populations would retain higher levels of genetic variation in fitness at mutation-selection balance was not supported. Finally, diversity may have been maintained by negative-frequency-dependent selection, the fitness of a genotype being highest when rare (because resources are most abundant) but not when common (resources are rare and competition intense) (92). Invasion experiments of each clone against the population from which it came revealed a small but statistically significant fitness advantage when rare, consistent with the operation of negative-frequency-dependent selection through cross-feeding interactions (111). These results suggest that, given sufficient time, selection may lead to the evolution of new kinds of interactions among genotypes even in the simplest of environments, a phenomenon discussed in more detail below.

Complex Environments Composed of Many Niches

A heterogeneous environment is one composed of many niches that may vary in either space or time, and at different scales relative to the generation time of the organism concerned (64). The connection between environmental heterogeneity and diversity has a long history in ecology and population genetics (85, 86, 109), although direct tests of theory have been comparatively recent (64).

Evolutionary theory predicts that in a spatially heterogeneous environment, selection favors the emergence of ecological specialists, different types being adapted to different niches. Ecological specialists by definition trade off enhanced competitive advantage in one niche against reduced competitive ability in another. The existence of trade-offs prevents competitive exclusion by one type and renders coexistence of organisms possible (66, 104). The evolution of ecological specialization can be readily achieved in the laboratory by allowing a single founding population to grow and reproduce for many generations in contrasting environments. Bell and Reboud (6, 108), for example, founded two populations from a single genotype of the unicellular chlorophyte *Chlamydomonas reinhardtii* that were serially propagated for approximately 1000 generations either as phototrophs in the light or as heterotrophs in the dark in an environment supplemented with

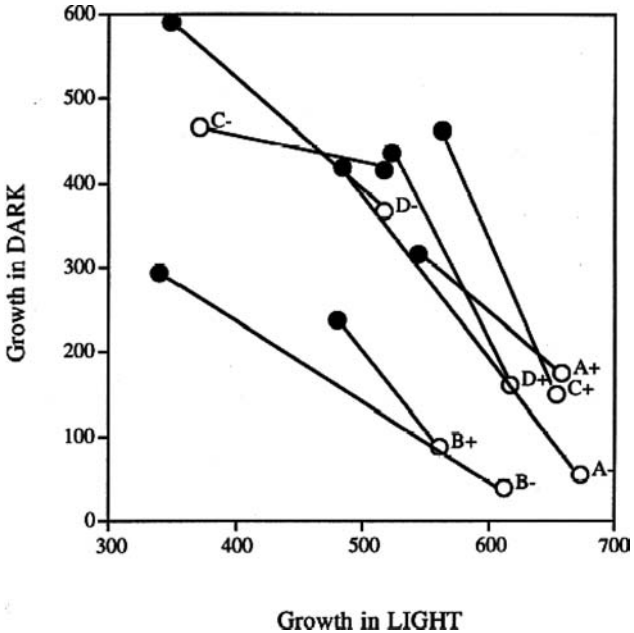

Figure 3 The evolution of ecological specialists through selection in different environments. Each pair of points connected by a line represents the fitness of a single genotype of *Chlamydomonas* in the light (*x-axis*) and in the dark (*y-axis*) that has been selected in the light (*open circles*) and in the dark (*filled circles*) for approximately 1000 generations. Note that specialization to one environment is accompanied by a fitness trade-off in the other environment. Reproduced with permission from Bell & Reboud (6).

organic carbon. The original genotype grew well in the light and relatively poorly in the dark. By the end of the experiment, the light lines showed little improvement in the light and lost the ability to grow in the dark, while the dark lines grew much better than the ancestor in the dark and relatively poorly in the light compared with the light lines (Figure 3). Trade-offs of this sort are commonly observed in laboratory experiments (39, 64). Indeed these sorts of fitness trade-offs across environments form the basis for the widespread use of vaccines, which have been adapted to laboratory conditions and therefore have lost the ability to cause disease in humans (39).

The evolution of ecological specialization and fitness trade-offs across environments is a necessary but not sufficient condition for the maintenance of diversity in a heterogeneous environment. This point is made by a second experiment by Reboud & Bell (108) in which the light and dark specialists were mixed together and then propagated for a further 200 generations in either spatially or temporally varying environments. Spatial variation was simulated by growing the mixed

population in the light and in the dark simultaneously, collecting samples from each, combining them together, and then redistributing them to light and dark flasks again. Temporal variation involved growing the original mixture first in one environment and then transferring a sample to the other environment for the next growth cycle. Theory predicts that spatial variation would be much more effective than temporal variation at maintaining diversity because it offers refuges for a rare type well-adapted to one of the environmental patches. In contrast, temporally varying environments offer no such refuge—the type favored being one that grows best across all conditions. The results bore out this prediction: Populations exposed to the spatially varying regime retained the mixture of light and dark specialists with which they were founded, whereas generalist types, capable of growing well under both light and dark conditions, evolved when the environment varied temporally. Thus the pattern of environmental variation—in time or in space—is central to the maintenance of diversity.

The combination of ecological specialization and spatial heterogeneity maintains diversity when otherwise it would be lost. Diversity in Reboud and Bell's experiment, for example, was rapidly lost when the mixed population was propagated solely in either the light or the dark, a result that has been observed many times previously (64). It is not clear from their experiment whether diversity would be ultimately lost in the spatially heterogeneous environment as well. In theory this need not happen. Models of selection in heterogeneous environments suggest that, under the right conditions, diversity can be permanently and stably maintained through negative-frequency-dependent selection (83, 93), the fitness of a genotype being higher when rare than when common. For this to happen, fitness trade-offs must exist, as before, and the population size must be regulated at the level of the local patch.

Evidence of trade-offs and the concomitant operation of negative-frequency-dependent selection has been detected and measured in laboratory populations of various microbes (8, 64, 80, 104). Rainey & Travisano (106) documented the emergence of diversity in spatially heterogeneous environments. Genetically identical founding populations of *Pseudomonas fluorescens* were propagated in heterogeneous (spatially structured) environments, afforded by incubating broth-containing microcosms without shaking, and homogeneous (spatially unstructured) environments, created by incubating identical microcosms under a continuously shaken regime. Diversity, in the form of niche-specialist genotypes, emerged rapidly in spatially heterogeneous microcosms, but not in spatially homogeneous microcosms. Taking ecologically diverse populations from spatially structured microcosms and propagating them under the spatially heterogeneous regime or the spatially homogeneous regime provided a test of the role of spatial heterogeneity in supporting diversity. Diversity was rapidly lost once heterogeneity was reduced, supporting the idea that heterogeneity itself is a primary cause of diversity and necessary for the continued maintenance of diversity.

In the *P. fluorescens* experiments, niche specialization and trade-offs are readily observable by eye (106); however, additional experiments were performed to

test the operation of negative-frequency-dependent selection—the mechanism expected to maintain diversity given the existence of obvious trade-offs. A series of pair-wise "invasion from rare" experiments showed that in most instances niche-specialist genotypes could increase in frequency relative to a common type, even though initially present at just 1/100 the density of the common type. The diversity is therefore protected and coexistence of genotypes is assured.

It is difficult to say with certainty whether conditions for the maintenance of diversity in laboratory populations also operate in the field, especially for microbial populations of which little is known about the dispersal rate and the degree of local adaptation. Clearly, most natural environments are often heterogeneous, although whether this heterogeneity occurs on the appropriate spatial scale for selection to maintain diversity remains to be seen. Some indication of the scale at which selection acts has been obtained by Belotte et al. (7), who isolated single colonies from soil samples collected from a 10×10 m plot in an old-growth forest in southern Quebec, Canada. The fitness of isolates was typically greatest when grown on soil-water extracts made from the same soil from which they had been isolated, and fitness was lowest when grown on soil extracts made from other samples, suggesting that the scale of local adaptation in these bacterial communities is on the order of 1 to 10 m. This and other recent studies suggest that spatial structure of the form that could stably maintain diversity is a common feature of microbial communities (23, 55, 118, 130, 131).

Complex Environments Created by the Growth of Competitors

It is reasonably easy to envisage GxE interaction for fitness arising through selection in response to different chemical resources or spatially distinct niches (84, 121). However, it is also possible for new sorts of interactions to arise as the result of the growth of genotypes themselves, even in the absence of physically or chemically distinct niches. The growth of one genotype creates a new niche for another genotype, and so diversity can be understood in much the same way as before, in which the number of niches determines the number of types supported in the community.

An example of such GxG interactions comes from the work of Adams and colleagues (56), who performed long-term selection experiments with chemostat-propagated populations of *E. coli* grown on a single limiting carbon source. Regular sampling from the chemostat populations revealed phenotypically distinct colony types on agar plates, indicating that the once genetically uniform populations had become polymorphic. Analysis of competitive interactions among genotypes showed that the polymorphism was stable and maintained by density-dependent processes. This surprising finding appeared at first to contradict the niche exclusion principle and prompted further physiological and genetic studies. These studies found evidence of resource partitioning of the limiting glucose resource by the numerically dominant genotype (110). Partitioning of glucose into

acetate and glycerol, metabolic byproducts arising from metabolism of glucose, provided ecological opportunity for the evolutionary emergence of mutants with enhanced capacities to metabolize these byproducts. Indeed, the variant types that arose (and arose repeatably; 128) had evolved, in one instance, an enhanced capacity to metabolize acetate and, in the other, an improved capacity to recover glycerol.

Diversity can also be maintained through GxG interactions that occur over small spatial scales and where strict competitive hierarchies are absent (29). Experimental studies of colicin-producing, colicin-resistant, and colicin-sensitive derivatives of *E. coli* (71) show that in an environment allowing for localized interactions, such as the surface of an agar plate, all three genotypes can be maintained. However, in an environment where opportunity for localized interactions is negligible, such as in a shaken (spatially homogeneous) broth culture, diversity is lost. Similar dynamics are likely to be widespread in microbial communities, such as biofilms (49, 50, 124), where opportunities exist for local interactions among individuals.

One important distinction between GxG interactions and GxE interactions is that the former can lead, at least in principle, to coevolution. Such interactions have the potential to increase or decrease diversity (18, 20, 22), with the outcome being dynamic and difficult to predict. The clearest examples of coevolution between microbial genotypes come from studies of predator-prey and host-parasite interactions (13, 14, 34, 59). In principle, GxG interactions may lead to coevolution between competitors as well, but, to our knowledge, this has not yet been documented in experimental populations of microbes.

Complex Environments Created Through the Growth of Predators

Our discussion has so far focused on explanations for diversity in simple communities composed of different genotypes or species competing for similar resources. Natural communities may be much more complex, however, with many trophic levels. These more complex communities can be understood as a special case of GxG interactions, in which the ecological interactions between predator and prey are severely asymmetric: positive for the predator and negative for the prey. This represents a strong selective pressure on the prey population to evolve means of escaping predation. Several experiments with bacterial prey and their phage predators have documented the spontaneous evolution of phage-resistant bacteria from a population of sensitive types (9, 21, 81). Interestingly, both sensitive and resistant prey genotypes can coexist in the presence of phage if there is a trade-off between growth rate and susceptibility to predation: Resistant genotypes avoid predation but grow slower than sensitive genotypes in the absence of phage. The magnitude of this trade-off can have profound impacts on the relative frequencies of the different types in the community (9). Extrinsic features of the environment can also determine the variety of trophic interactions in a community. These issues are explored in more detail by Jessup et al. (63).

Patterns of Diversity in a Collection of Communities

Diversity in metazoan communities often follows regular patterns across environmental gradients in nature (109), and there seems little reason to expect that the same might not be true in microbial communities. In a recent review, Horner-Devine et al. (58) provide evidence to support this contention. They show that molecular variation in bacterial communities often changes in regular ways across different kinds of habitats, within habitats that differ in their structural diversity, and across gradients of primary productivity and disturbance. These results need to be interpreted with caution because they are often based on molecular variation that may not always accurately reflect the quantity of genetic variation in fitness. Still, the fact that some of these patterns bear a striking resemblance to those observed in metazoans suggests that similar processes may be at work.

Our own work has explored two of these patterns: those caused by alterations in productivity (67) and disturbance (12). Along gradients of both factors, species diversity is typically humped-shaped, with a peak at intermediate rates of production and disturbance. Traditionally, ecologists had thought that different mechanisms were responsible for the two patterns. By examining diversity of *P. fluorescens* populations cultured along gradients of both productivity and disturbance, it appears that both patterns may be underlain by the same mechanism, namely competition among niche specialists in a heterogeneous environment. The key features determining diversity in this system are diversifying selection—different niche specialists favored in different niches—and the relative fraction of individuals contributed by each niche to the total population. Diversity can only be maintained if each niche contributes similar numbers of individuals to the total population; if one niche produces many more individuals than the other, diversity cannot be maintained. We have shown, furthermore, that the same mechanism may govern the level of diversity achieved during adaptive radiation, in which a single lineage diversifies to form a range of niche specialists (68), suggesting that ecological factors can act as major constraints on diversification over evolutionary timescales.

The *Pseudomonas* experiments lend support to the idea that environmental structure is important in determining the level of diversity achieved not only within a community, but also across communities in a landscape. These patterns may be further modulated by a variety of factors such as parasites (10, 13), predators (9, 69), migration among populations in a landscape (27), and the contingent nature of the interactions among types underlying the assembly of communities (46). The extent to which these factors determine patterns of diversity in natural microbial communities remains an empirical issue.

THE GENETIC CAUSES OF DIVERSITY IN AN ECOLOGICAL CONTEXT

The ultimate source of all genetic variation is, of course, mutation. New genetic variants may also be introduced into the community through migration or created by recombination. These mechanisms serve to increase the amount of heritable

variation from which natural selection sorts under different ecological conditions and so can be readily understood within the framework outlined above. This approach is not very useful, however, for understanding in detail the genetic mechanisms underlying the origin of diversity or the impact these mechanisms have on the range of variation available to natural selection within a given ecological context. We turn to these problems in this section.

Genetic Mechanisms Underlying Diversification Through Adaptive Radiation

The vast majority of phenotypic and ecological diversity on the planet has arisen during successive adaptive radiations, that is, periods in which a single lineage rapidly diversifies to generate multiple niche-specialist types (35, 77, 113, 114). Microbiologists tend not to think of microorganisms as undergoing adaptive radiation, but there is no reason to exclude them from this general statement—in fact the rapid generation times and large population sizes characteristic of many microbial populations suggest that microorganisms may be particularly prone to bouts of adaptive radiation (6, 90, 106, 127).

Diversification during adaptive radiation requires that a lineage first gain access to a niche in which it has a selective advantage over the ancestral type. The phenotypic changes favored by selection during these early stages of adaptive evolution typically involve increases in levels of expression of enzymes that are initially limiting to growth in the novel environment. These higher levels of expression allow the population size to increase, which then sets the stage for subsequent divergence through modification and refinement of the existing genome. Uncovering the genetic changes responsible for diversification thus represents a special case of the more general problem of the genetics of adaptive evolution and phenotypic innovation (19, 41, 78, 103, 120). Here we briefly mention two mechanisms responsible for the initial invasion of a novel niche, gene duplication and changes in gene regulation.

Sonti & Roth (117) have documented the selection of stable duplications in the *araE* locus of *Salmonella typhimurium*, a gene coding for a permease used to import arabinose, sorbitol, and malate. Under standard conditions, duplications in *araE* occur at rates of about 10^{-3} to 10^{-4}, but they were elevated approximately 2000-fold when cultures were grown under arabinose limitation on chemostats for roughly 200 generations. Similar results were obtained when cultures were selected under sorbitol and malate limitation as well. These experiments show that duplications, particularly in genes involved in nutrient acquisition, are favored under selective growth (1, 57).

Once duplicate genes are fixed in a population, one copy is free to accumulate mutations while the other retains its original function. This permits the lineage possessing the duplication to take advantage of new and unexploited resources while not forgoing the ability to grow on the original substrate. This may not always be the case; one of the duplicated copies may be lost, or mutations may occur in both thus destroying complementary functions by a process known as

subfunctionalization (53, 89). Nevertheless, gene duplication has been implicated as a pervasive mechanism to explain the evolution of novel metabolic capabilities (87), the redundancy of eukaryotic genomes (51), and the evolution of novel morphological features in plants and animals (103).

The second mechanism facilitating access to novel environments has its roots in developmental genetics, in which molecular analyses have provided compelling evidence that phenotypic innovations often arise through changes to regulatory rather than structural genes (120). This is because it is easier for mutations to generate variation in the quantity of enzyme production, which is often controlled by regulatory genes, rather than in the specificity of those enzymes themselves. Such mutations are also less likely to have deleterious pleiotropic effects (120).

An example of this comes from studies of the evolution of novel substrate use in bacteria. Lin et al. (87) review a series of experiments stemming from the pioneering studies of Wu and Mortlock (cited in Reference 87) on experimental evolution of xylitol metabolism by *Klebsiella aerogenes*; xylitol being a substrate not normally found in nature and on which wild-type strains cannot grow. Selection in liquid medium containing xylitol as a sole carbon and energy source generated a series of mutants (X1–X3) with increasing growth rates. Biochemical analysis of the first mutant to appear, X1, showed that it constitutively expressed ribose dehydrogenase (RDH), which is normally an inducible enzyme that hydrolyzes ribitol into ribulose. RDH from X2, a second mutant derived from X1, is not only constitutively expressed on xylitol but shows structural alterations to the enzyme that increase its affinity for xylitol. Thus the evolution of novelty in this system involves the deregulation of a pre-existing enzyme and the subsequent modification of its structural properties. Changes in levels of enzyme expression are also responsible for the evolution of acetate cross-feeding mutants of *E. coli* (128) and niche specialization in the model *P. fluorescens* adaptive radiation (105, 119).

Fitness Effects of Mutations

The process of diversification characteristic of adaptive radiation involves adaptation to novel conditions of growth, which itself depends on the supply of new, beneficial mutations. Mutations arise constantly by a variety of mechanisms (92), but most of these are deleterious and never achieve high frequency in a population. It is the much smaller class of beneficial mutations that provides the fuel for adaptive evolution.

One approach to studying beneficial mutations is to characterize them through a combination of laboratory selection and genetic analysis (16, 17, 25, 41). Bull et al. (16) identified the genetic changes in the bacteriophage ϕX174 that permit growth at high temperature after a single round of selection. Two of the three major classes of mutation occurred at different locations within the capsid protein, and the third concerned a gene expressing a protein involved in the development of the mature virion. The precise biochemical and physiological effects of these mutations are not known, but they seem to be involved in maintaining the stability of the immature and mature virus particle at high temperature (36).

That these mutations were the ones responsible for adaptation was confirmed by estimating the fitness of the different genotypes relative to the ancestor from which they were derived. All three mutants conferred large fitness advantages relative to the ancestor at the high temperature. However, adaptation to this novel environment came at a cost: The mutants had reduced fitness (relative to the ancestor) in the low temperature ancestral environment. This cost (in which a mutation is beneficial in one environment but deleterious in another) is known as antagonistic pleiotropy and forms one of two potential costs of adaptation—the other arising from mutations that are neutral in the environment of selection but deleterious elsewhere (26, 91). Finally, the beneficial effect of the mutations depended on the genetic background in which they occurred. The original mutations were recovered from strains that were not well adapted to growth in *E. coli*. When the mutations were transferred into a genetic background that was well adapted to *E. coli*, the gains in fitness were substantially smaller. Thus the fitness of a given mutation is contingent on the genetic background in which it occurs.

A second approach to studying beneficial mutations is to focus on the statistical properties of the variation among the mutations without regard to their mechanistic bases. Fisher's geometric model of adaptive evolution predicts that the distribution of fitness effects among beneficial mutations, both new and those ultimately fixed by selection, follows a negative exponential, with many mutations of small effect and few of large effect (45, 99). Imagine a population located at some distance from a fitness optimum, represented in Figure 4 by the intersection of the

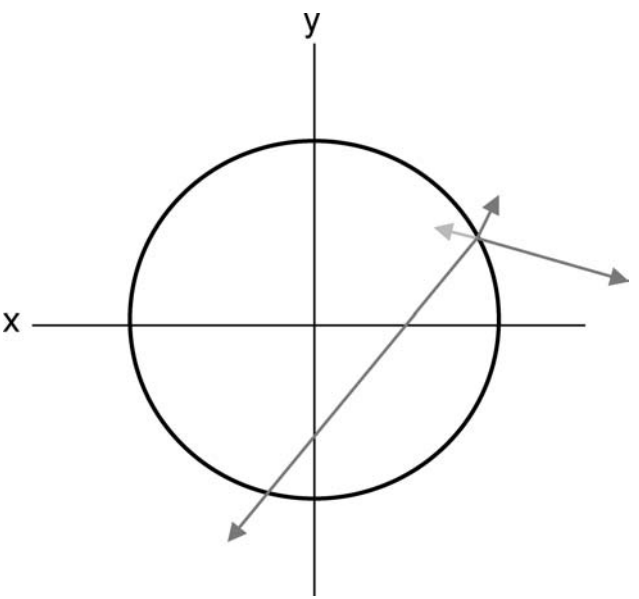

Figure 4 A schematic of Fisher's geometric model of adaptation. See text for a description.

x- and y-axes. Mutations of variable effect on the phenotype arise at random and are represented by vectors of any direction and magnitude in space that have the location of the original population as their origin. Only those mutations that bring the population closer to the fitness optimum are favored by natural selection. Large mutations stand a greater chance of taking the population farther away from the fitness optimum, or even overshooting it, and therefore are unlikely to be viable. Small mutations, however, have a higher probability of bringing the population closer to the optimum and therefore are more likely to contribute to adaptation.

To study experimentally the distribution of fitness effects among beneficial mutations, Imhof & Schlotterer (62) followed the evolution of 10 replicate *E. coli* populations for 1000 generations starting from a single ancestral strain containing a highly mutable microsatellite locus from *Arabidopsis thaliana* contained on a plasmid. Population expansion generated a variety of microsatellite alleles that were neutral with respect to the fitness of *E. coli* cells. Selective sweeps caused by beneficial mutations were detected by monitoring the frequency of specific microsatellite alleles to which particular beneficial mutations were genetically linked. Isolation of the strains bearing the putative beneficial mutations and analysis of their fitness relative to the population from which they were isolated (as opposed to the usual practice of competing them against the unevolved ancestor) provided an estimate of the selective advantage of each newly risen mutant. Taken together, the distribution of fitnesses among these beneficial mutations was not statistically different from a negative exponential distribution, as expected from theory.

CONCLUDING REMARKS: LIFE IN THE TANGLED BANK

> It is interesting to contemplate a tangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent upon each other in so complex a manner, have all been produced by laws acting around us.
>
> Charles Darwin (31, p. 429)

Microbial communities are highly diverse, a fact that demands both description and explanation. Much progress has been made over the past 15 to 20 years on the former; the experiments reviewed here take us some way toward the latter. From a purely ecological perspective, diversity is supported most readily in the presence of divergent natural selection: Different types are favored in different niches. The variety of niches available depends largely on the physical structure of the environment. Complex, heterogeneous environments provide more niches—and therefore are more likely to maintain diversity—than simpler environments with fewer niches. But the abiotic environment is not the sole determinant of niche space: The biotic environment is also relevant, with new ecological opportunities being continually created through the growth and activities of organisms.

There does not seem to be any serious limit to the ability of mutation to generate new variation upon which selection can act. Diversification in the face of ecological opportunity occurs through at least two mechanisms, gene duplication and deregulation of gene expression. Subsequent adaptation to novel environments can be interpreted both in mechanistic terms, as a consequence of the biochemistry and physiology of gene action, and in purely a statistical manner, in terms of the distribution of mutational effects.

That being said, some qualifying statements are necessary. Perhaps the most important is that we have ignored (quite deliberately) an important alternative explanation for diversity, namely that much of the variation observed in nature is neutral with respect to fitness, its fate determined by stochastic processes sorting among ecologically equivalent types. The neutral theory was originally developed to explain the greater than expected molecular variation revealed by studies of protein and DNA polymorphism (73), but it has recently been extended to cover species diversity in ecological communities (5, 60). We suspect that the unified neutral theory of biodiversity and biogeography (60) may have particular relevance to diversity in microbial communities. Note, however, that it cannot by definition explain the existence of genetic variation in fitness—the focus of this article. In this regard we need better estimates of the quantity of genetic variation in fitness that exists in natural communities (44).

A number of lines of evidence suggest that such variation in fitness exists in nature: The mere fact that some microbial species can be cultured and others cannot points to the existence of genetic variance in fitness for the conditions of culture. Schmidt and colleagues (74) provided evidence that ribosomal copy number influences the structure of microbial populations in soil. In addition, the composition of microbial communities typically changes along environmental gradients, such as temperature and depth in hot springs (130), different soil and moisture types (11, 112) and geographical locations (55, 131), length of the gastrointestinal tract (15), and space and time within oral cavities (75), suggesting that selection in these different environments is often strong enough to lead to ecological specialization. Direct, experimental evidence demonstrating adaptation to these different environments, and the scale at which this occurs, can be obtained through reciprocal transplant or explant experiments (7). Evidence can also be obtained by careful manipulation and monitoring of the response of natural microbial populations to perturbations (74).

By way of further qualification, we recognize that we have not considered the effects of dispersal and recombination (gene flow) on the maintenance of diversity in microbial populations. These are of particular relevance, as both factors limit the ability of selection to maintain diversity in heterogeneous environments and both dispersal and recombination are undoubtedly significant factors in microbial communities. The trouble is we have little idea over what scales these operate or the full magnitude of their effects (24). Nevertheless, there is scope for incorporating these factors into future experiments.

Despite these caveats there can be little doubt that microbes, on account of their rapid generation times and large population sizes, have a remarkable capacity to

evolve and diversify through natural selection. This demands that we pay attention to the evolutionary context of microbes and the populations and communities of which they are part. Microbial communities are evolving entities whose components are genotypes and species and whose driving mechanisms are the interactions among them. These interactions may take on any form, from antagonistic to beneficial, and can themselves evolve, sometimes fortuitously and unexpectedly. For example, consider the relationship between bacterial pathogens and disease. Single cells rarely ever cause disease—it is a property of populations of cells and more usually communities. Disease symptoms typically manifest once pathogen populations reach certain thresholds, and the likelihood that these are reached depends on the nature of the interactions (antagonistic or synergistic) among individuals and populations that comprise the community. Moreover, the composition of the community, and therefore the severity of disease, can be modified by ecological factors, such as nutrient supply and biomass disturbance, that in turn are influenced by prevailing therapeutic regimes. Of course the severity of disease is also influenced by evolution within the pathogen population occurring within the ecological context defined by the host itself.

That we become more conversant with the central concepts of ecology and evolution and begin to place our detailed molecular understanding of the lives of microbes within this context is crucial. The techniques required to do this are essentially the same as those introduced by Dallinger well over a hundred years ago and require only that we pay closer attention to the interactions among genotypes and species and their consequences at the population and community levels. We suspect that when more is known about the variety of these interactions and the complexity of the habitats in which they occur in nature, the resulting picture of life in microbial communities will bear a striking resemblance to the one Darwin gave of his tangled bank.

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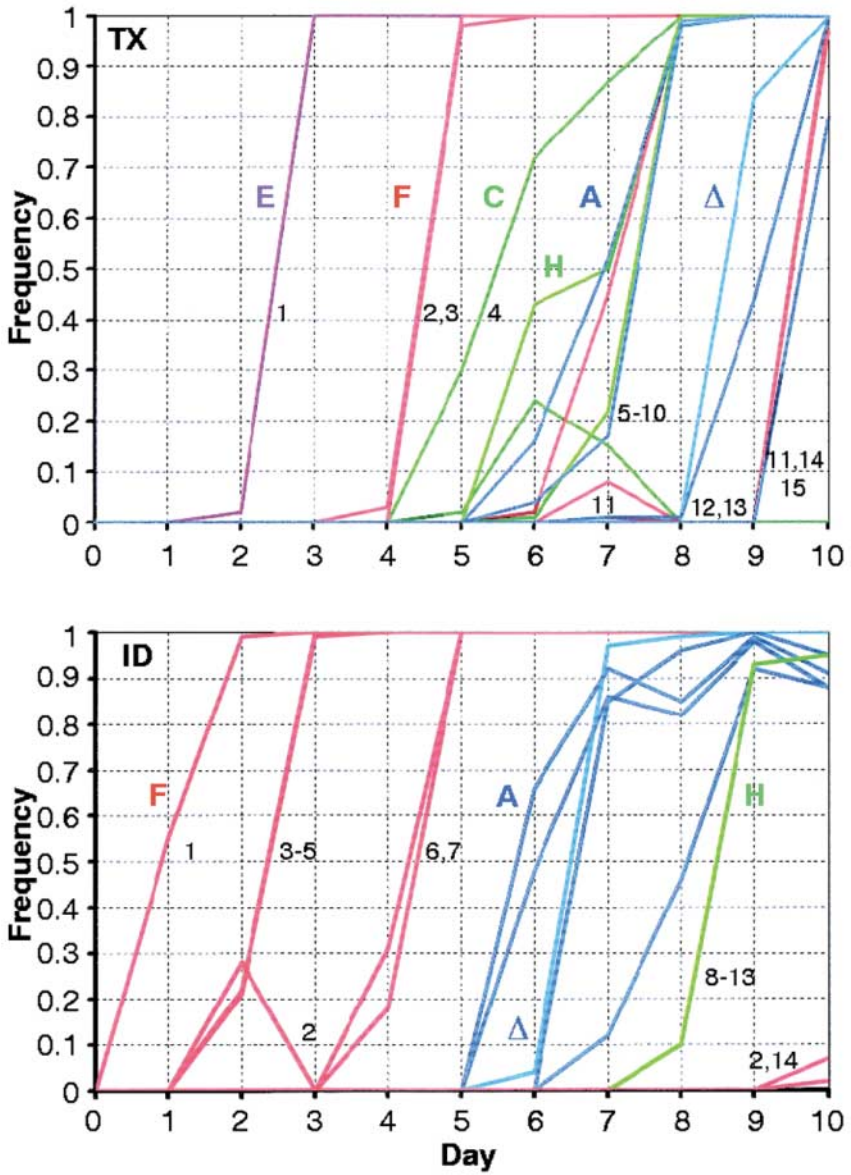

Figure 2 Molecular evolution of beneficial phage mutants in two populations, TX and ID. Lines and letters represent the frequency of novel mutants on a given day. Numbers refer to the order in which each mutant appeared. Note that a single genotype tends to dominate the population at any given time. Reproduced with permission from Wichman et al. (133).