Adaptive radiation in microbial microcosms

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Introduction

One of the most prominent features in the history of life is adaptive radiation, the simultaneous evolution of genetic and ecological diversity in a single lineage. Celebrated examples of adaptive radiation include the Cambrian explosion of metazoans (Gould, 1989), the diversification of cichlids in the Great Lakes of Africa (Fryer & Iles, 1972), and the radiation of Darwin’s finches in the Galápagos islands (Grant, 1986). A large body of theory argues that adaptive radiation is the ultimate result of divergent selection for specialization on alternative resources (Schluter, 2000). According to this theory, divergent selection results from differences in the resources available among habitats (Dobzhansky, 1937; Mayr, 1963) and from competition for resources within the same habitat (Maynard Smith, 1966; Slatkin, 1980; Doebeli, 1996; Geritz et al., 1998; Kondrashov & Kondrashov, 1999; Doebeli & Dieckmann, 2000). Recently, Schluter (2000) compiled a comprehensive review of tests of this theory in natural populations of macro-organisms. The goal of this paper is to provide a review of adaptive radiation and the evolution of resource specialization in microbial microcosms.

The rationale for the use of microbial microcosms as model systems in evolution is that mechanisms of evolution can only be elucidated through the use of experiments. Laboratory populations of microorganisms are an ideal system for such experiments. The short generation times (i.e. hours) and large population sizes (usually >10⁶) of laboratory populations of microorganisms allow for rapid evolution under tightly controlled conditions. In microbial microcosms, it is often possible to directly observe adaptive radiation on a time scale of weeks to months. The ease with which both organisms and environments can be manipulated in the lab allows for explicit tests of hypotheses concerning both the causes and consequences of diversification and specialization. Performing similar experiments in the field with macro-organisms is possible, but difficult (e.g. Schluter, 1994; Bolnick, 2004).

In 1990, Daniel Dykhuizen published a review of studies of natural selection in experimental microbial systems. At the time, so few experiments had studied adaptive radiation that it did not merit a complete section in his review. The experimental evolution literature has grown rapidly (for general reviews, consult Bell, 1997; Ebert, 1998; Kassen, 2002; Elena & Lenski, 2003) and microbial studies of adaptive radiation and resource...
specialization have been previously reviewed (Rainey et al., 2000; Travisano & Rainey, 2000; Kassen & Rainey, 2004). In this review, I attempt to provide a broad discussion of the contribution of microbial selection experiments to our understanding of resource specialization and adaptive radiation. I begin by discussing the ecological causes of adaptive radiation. This is followed by a discussion of the population and molecular genetics of resource specialization in microbes. I conclude by summarizing the microbial literature and by suggesting future avenues of research with microbes that could make important contributions to our understanding of adaptive radiation.

The ecology of adaptive radiation

What are the causes of adaptive radiation? According to the ‘ecological theory’ (Schluter, 2000), adaptive radiation is the result of divergent natural selection for resource specialization in heterogeneous environments containing a diversity of resources. The underlying premise of models that explore the evolutionary consequences of resource competition is that competition for commonly exploited resources generates selection for the exploitation of underutilized resources. Provided that trade-offs exist in the ability to exploit alternative resources, resource competition will, therefore, generate divergent selection for specialization on alternative resources. In these models, divergent selection in asexual organisms results in adaptive radiation into specialist lineages that exploit different resources or niches (Geritz et al., 1998; Dieckmann & Doebeli, 2000). The ecological causes of divergent selection are the same in sexual organisms (Slatkin, 1980; see review in Taper & Case, 1992; Doebeli, 1996), but speciation will only occur if assortative mating evolves within emerging specialist lineages (Maynard Smith, 1966; Dieckmann & Doebeli, 1999; Kondrashov & Kondrashov, 1999). A single generalist, well adapted to consume all of the available resources, is expected to evolve in both sexual and asexual organisms if trade-offs do not exist in the ability to exploit different resources (Via & Lande, 1985). The critical prediction of the ecological theory of adaptive radiation is that ecological and genetic diversification is a general feature of evolution in heterogeneous environments. The appropriate experimental test of this hypothesis, therefore, is to introduce an ancestral microbial genotype into a novel environment containing a diversity of resources and to assay for the evolution of ecological and genetic diversity in evolved genotypes derived from the ancestral clone.

An elegant demonstration of adaptive radiation in a microbial microcosm comes from the work of Rainey & Travisano (1998). A broth-colonizing genotype of the bacterium *Pseudomonas fluorescens* was inoculated into microcosms that were incubated without physical disturbance or with continuous shaking. Microcosms were destructively vortexed and dilute samples were spread on agar plates at given time intervals. Plotted points show the mean density (±SEM) of colony morph variants (SM: circles; WS: triangles; FS: squares) in undisturbed (a) and shaken (b) microcosms. (c) Colony morphology and spatial distribution in unshaken microcosms of the three classes of morph variant. Figures reproduced from Rainey & Travisano (1998) with permission from the authors and Nature Publishing Group.

![Fig. 1 Adaptive radiation in *Pseudomonas*. A single clone of *P. fluorescens* was inoculated into microcosms that were incubated without physical disturbance or with continuous shaking. Microcosms were destructively vortexed and dilute samples were spread on agar plates at given time intervals. Plotted points show the mean density (±SEM) of colony morph variants (SM: circles; WS: triangles; FS: squares) in undisturbed (a) and shaken (b) microcosms. (c) Colony morphology and spatial distribution in unshaken microcosms of the three classes of morph variant. Figures reproduced from Rainey & Travisano (1998) with permission from the authors and Nature Publishing Group.](image-url)
frequency-dependent selection stemming from trade-offs in the ability of metabolic variants to exploit different substrates in the broth. A similar radiation into metabolic variants occurred when *Escherichia coli* was selected in spatially homogeneous microcosms containing a diversity of sugars (Friesen et al., 2004).

If resource heterogeneity were the ultimate cause of diversification in microbial microcosms, we would expect that adaptive radiation should not occur in homogeneous microcosms containing a single resource. A number of microbial selection experiments have reported results that are in good agreement with this prediction (see reviews in Dykhuizen, 1990; Elena & Lenski, 2003), the notable exception to this being the repeated finding that populations of *E. coli* diversify when selected in microcosms containing glucose as the sole carbon substrate (Helling et al., 1987; Rosenzweig et al., 1994; Rozen & Lenski, 2000). Helling et al. (1987) originally reported diversification of a chemostat population of *E. coli* into genotypes that formed either large or small colonies on agar plates. Subsequent physiological and biochemical studies (Rosenzweig et al., 1994) established that small morph colonies rapidly consumed glucose, but secreted large quantities of incompletely oxidized intermediates of glucose metabolism, such as acetate and glycerol. Large morph colonies, on the other hand, slowly consumed glucose but were able to scavenge the waste products produced by small morph colonies. The cross-feeding interaction between large and small morph colonies generated negative frequency-dependent selection that allowed both morphs to co-exist. The evolution of cross feeding illustrates how the exploitation of a single resource can generate a variety of alternative resources that can support specialist populations.

The genetic basis of adaptive radiation

Although ecological forces generate the selective pressure required for the emergence of diversity, the ultimate source of heritable phenotypic variation is mutation. Studies of the genetic basis of adaptive radiation focus on three principle questions that involve different scales of genetic investigation. At a molecular level, there is interest in the nature of mutations that generate resource specialist phenotypes. At the population genetic level, there is interest in the genetic basis of trade-offs in the ability to exploit different resources. Above the scope of population genetics, interest persists in the repeatability of adaptive radiation. This question is of general interest to evolutionary biology, because it lends insight into the relative importance of chance, historical contingency, and natural selection in shaping the genetic outcome of adaptation.

Molecular genetics of resource specialization

Although we need not understand what kinds of mutations generate specialist phenotypes to understand why diversification occurs, an understanding of the genetic basis of niche specialization allows us to understand how diversification occurs at a genetic level. Unlike the ecological theory of the causes of adaptive radiation, we have no general framework for predicting what kinds of mutations (i.e. insertions, deletions) generate specialist phenotypes and which categories of loci are involved (i.e. structural loci or regulatory loci). The comparative approach to this problem involves comparing rates and patterns of sequence evolution in lineages that are known to have undergone an adaptive radiation. The experimental approach involves a direct determination of the relevant loci that can be mutated to produce a given specialist phenotype. Microbes are excellent models to study the genetic basis of adaptation because of their small genomes that can be easily manipulated.

The physiological and genetic mechanisms of microbial specialization on novel resources were the subject of considerable investigation by biochemists and geneticists in the 1970s and 1980s (see review in Mortlock, 1984b). The general protocol for these experiments was to introduce a large population of a chemically mutagenized ancestral genotype into a chemostat containing a novel carbon compound as the sole source of available carbon. Mutants capable of growth on the novel substrate were then isolated and the process of chemical mutagenesis and natural selection was repeated until a successive series of genotypes with improved growth on the novel substrate were obtained. The general conclusion of this fascinating and often neglected literature is that the critical step in adaptation to a new carbon source involves regulatory mutations that increase the production of existing catabolic genes. Typically, this involves the constitutive expression of catabolic enzymes with a low affinity for the novel substrate, but high affinity for structurally similar compounds (Clarke, 1984; Hall, 1984; Lin & Wu, 1984; Mortlock, 1984a,c). Subsequent adaptation can occur by increasing the expression of these exapted catabolic enzymes through gene duplication (Hartley, 1984). Alternatively, adaptation may occur via structural mutations that increase the affinity of exapted enzymes for their novel substrate (Clarke, 1984; Mortlock, 1984c). An excellent example of this process comes from the work of Mortlock (1984c), who studied the evolution of metabolic pathways for novel pentitol sugars in *Klebsiella aerogenes*. The first step in the degradation of two novel pentitols, xylitol and L-arabitol, can be catalyzed by ribitol dehydrogenase (RDH), an enzyme with a high affinity for the pentitol sugar ribitol. Growth on xylitol and L-arabitol is normally not possible, because the expression of RDH is repressed in the absence of ribitol. Initial adaptation to novel pentitols occurs by mutations that result in the constitutive expression of RDH. Improved growth on novel pentitols occurs primarily as a result of structural mutations that improve the affinity of RDH for either xylitol or L-arabitol.
Conventional selection experiments that have extended this research programme by examining the mechanisms by which increased specialization evolves on carbon substrates that microbes have at least some rudimentary ability to metabolize. This literature also demonstrates that changes in levels of gene expression are a prominent feature of the evolution of resource specialization (Brown et al., 1998; Treves et al., 1998; Cooper et al., 2003). Evidence that regulatory mutations are a general feature of the evolution of ecological specialization, and not simply a feature of carbon substrate specialization, comes from studies of spatial specialization in P. fluorescens. WS morphs colonize the air-broth interface of undisturbed microcosms as a result of the production of an acetylated cellulose-like polymer (Spiers et al., 2003). The expression of this phenotype depends on the constitutive expression of a cellulose biosynthetic operon, wss, that contains structural genes that code for biosynthetic proteins as well as a regulatory locus (Spiers et al., 2002).

In summary, the available evidence suggests that resource specialization in microbes is primarily attributable to mutations that de-regulate the expression of existing operons. At present there is not enough data on the genetic basis of niche specialization in macroorganisms to allow us to determine the generality of this result, although the limited evidence from both plants (Barrier et al., 2001) and animals (Shapiro et al., 2004) implies that regulatory mutations also play a role in adaptive radiation in multicellular eukaryotes.

The population genetics of ecological specialization

Although ecological opportunity is required to generate selection for specialization on alternative resources, the response to selection will only result in diversification if genetic trade-offs exist in the ability of genotypes (and not individuals) to exploit different resources. In other words, the relevant evolutionary trade-offs involve a negative genetic correlation of fitness on alternative resources. Such trade-offs can evolve as a direct consequence of specialization if the same mutations have opposing effects on the ability to compete for alternative resources as a result of antagonistic pleiotropy (Levins, 1968). Alternatively, it is possible for resource trade-offs to evolve as an indirect consequence of specialization. In the short-term, independent adaptation to different resources in different specialist lineages is expected to lead to the evolution of fitness trade-offs. In the long term, these trade-offs are expected to be magnified by a cost of specialization resulting from the accumulation of conditionally deleterious mutations that impair the ability to compete for unused resources (Kawecki, 1994; Fry, 1996; Whitlock, 1996; Kawecki et al., 1997). A schematic of these two accounts of the evolution of trade-offs is shown in Fig. 2.

Although a large number of microbial selection experiments have reported that resource specialization is associated with trade-offs that reflect a cost of specialization (Bell & Reboud, 1997; Reboud & Bell, 1997; Travisano, 1997; Ebert, 1998; Weaver et al., 1999; Cooper & Lenski, 2000; Turner & Elena, 2000; MacLean & Bell, 2002; MacLean et al., 2004), relatively few studies have been able to determine the extent to which this cost reflects antagonistic pleiotropy. Cooper & Lenski (2000) found that the evolution of specialization on glucose in E. coli was accompanied by a cost, in terms of the inability to consume a wide range of unused carbon sources. Most of the cost of specialization evolved early in this experiment when adaptation to glucose was occurring rapidly, as predicted by the hypothesis of antagonistic pleiotropy. Critically, lines with elevated mutation rates did not express a greater cost of specialization than wild-type lines, demonstrating that the accumulation of

Fig. 2 The evolution of fitness trade-offs. Plotted points show the fitness of two populations selected for specialization on two hypothetical resources, A (triangles) and B (squares) at two different time points (open and closed symbols). Initially, both populations have equal fitness on both resources. In (a), specialization on resources A and B are not accompanied by an immediate cost (open symbols). A cost of specialization evolves in the long-term as a result of the accumulation of conditionally deleterious mutations that reduce fitness on alternative resources (filled symbols). In (b), specialization on both resources is accompanied by a direct cost as a result of antagonistic pleiotropy (open symbols). This cost is magnified after continued selection for specialization as a result of both pleiotropy and mutation accumulation (filled symbols).
conditionally deleterious mutations was not responsible for the cost of specialization. Similar techniques were recently used to investigate costs associated with the evolution of spatial specialization in Pseudomonas (MacLean et al., 2004). WS morph genotypes that initially colonized the air-broth interface of unshaken microcosms lost the ability to grow on a wide range of carbon sources. This cost could be attributed to antagonistic pleiotropy, because reversion of the WS phenotype to the ancestral broth-colonizing phenotype was associated with a complete disappearance of the cost of specialization. In contrast to the results of Cooper and Lenski, where costs of glucose specialization continued to increase over a period of 20,000 generations, the cost associated with WS almost completely disappeared after ≈100 generations of continued selection.

Both of these studies demonstrate that antagonistic pleiotropy can contribute to the evolution of trade-offs through a direct cost of specialization. The difference between these studies lies in the relationship between the fitness of specialists and the cost of specialization. The pleiotropic cost of glucose specialization escalates through time because mutations that impair the ability to consume alternative carbon sources directly cause an increase in the fitness of glucose specialists of E. coli (for example, Cooper et al., 2001). In this case, a positive genetic correlation exists between the costs and benefits of specialization. Catabolic defects that were associated with mutations that generated the WS phenotype were an indirect consequence, and not a direct cause, of increased fitness. In other words, the benefit of WS stems from the ability to colonize the oxygen-rich surface layer of undisturbed microcosms, and not from impaired metabolism. The cost of WS diminished after continued selection because mutations that restored normal metabolism caused an increase in the fitness of WS (MacLean et al., 2004). In this case, a negative genetic correlation exists between the costs and benefits of specialization.

The general principle that emerges from studies of the evolution of pleiotropic fitness trade-offs in E. coli and Pseudomonas is that antagonistic pleiotropy can only make a long-term contribution to trade-offs when a positive genetic correlation exists between the fitness of specialists and the cost of specialization. How common is this relationship between the costs and benefits of specialization? MacLean & Bell (2002) assayed the evolutionary consequences of specialization on 95 different carbon sources, providing an extensive data set with which to address this question. In this experiment, most specialist populations lost the ability to grow on several carbon substrates, demonstrating the existence of a cost of specialization. A positive genetic correlation between fitness and the cost of specialization was found in only 37 of the 95 selection lines, implying that antagonistic pleiotropy cannot be invoked as a general explanation for long-term maintenance of resource trade-offs in this large-scale experiment. Transient pleiotropic trade-offs that disappear during the evolution specialization as a result of compensatory adaptation may also explain why other selection experiments have failed to detect direct costs associated with resource specialization (Bennett & Lenski, 1993; Korona, 1996; Travisano, 1997; Riley et al., 2001).

Models of the evolution of resource specialization assume that pleiotropic trade-offs between specializations on alternative resources are either ubiquitous (Levins, 1968; Roughgarden, 1972; Lynch & Gabriel, 1987; Wilson & Yoshimura, 1994) or nonexistent (Kawecki, 1994, 1995; Fry, 1996; Whitlock, 1996; Kawecki et al., 1997). Microbial selection experiments demonstrate that neither of these assumptions is correct; pleiotropic trade-offs between the exploitation of alternative resources exist, but pleiotropy is by no means ubiquitous. One possible explanation for this result is that most selection experiments assay for costs of specialization after hundreds or thousands of generations of selection for specialization. It is conceivable that these assays do not detect pleiotropic costs of specialization because many of these costs disappear rapidly during the evolution of specialization, as in the example of metabolic costs associated with the WS morph of Pseudomonas.

**The repeatability of adaptive radiation**

How repeatable a process is adaptive radiation? Does each instance of adaptive radiation represent a unique evolutionary outcome, or do adaptive radiations follow a predictable set of ‘rules’? Comparative studies in a wide variety of taxa document the repeated evolution of similar niche-specialist phenotypes or ‘ecomorphs’ in replicate adaptive radiations that have occurred in similar environments (Losos et al., 1998; Rüther et al., 1999). The repeatable evolution of niche-specialist phenotypes is also a common feature of adaptive radiations in microbial microcosms (Rainey & Travisano, 1998; Treves et al., 1998). If the phenotypic outcome of adaptive radiation is broadly reproducible, the genotypic outcome of adaptive radiation must depend on the complexity of the relationship between genotype and phenotype: if only a few genotypes can produce a given phenotype, adaptive radiation will be reproducible at a genotypic level; if many genotypes can produce similar phenotypes, then the genotypic basis of adaptive radiation might be largely determined by chance and contingency.

Two approaches can be used to investigate the genetic basis of adaptation. The most powerful method is to sequence the mutations that give rise to specialist phenotypes. Unfortunately, very little data is currently available on the genetic differences between similar specialists that evolve during replicate radiations. An alternative approach is to infer the genetic basis of adaptive radiation by using only phenotypic traits. This approach, which has been used quite extensively in selection experiments with *Drosophila* (Cohan & Hoffmann,
were selected on a range of carbon compounds (Fong et al. 2001) or growth on the substrate a line was used during replicate instances of adaptive radiation, such that this technique has only limited ability to infer the genetic basis of adaptive radiation. Several recent microbial experiments have overcome this limitation by using recently developed technology to measure hundreds of correlated responses to selection. Riley et al. (2001) selected a clone of the bacterium *Ralstonia* in either solid or liquid medium containing a single carbon source and then assayed correlated responses to selection using a wide variety of phenotypic traits. A similar assay was conducted by MacLean and Bell, who selected and assayed lines of *Pseudomonas* on 95 different carbon sources (MacLean & Bell, 2003). In both of these experiments, evolved lines exhibited similar direct responses to selection, measured as either competitive fitness in the environment a line evolved (Riley et al., 2001) or growth on the substrate a line was selected on (MacLean & Bell, 2003). In both cases, selection lines exhibited widely divergent correlated responses to selection among replicates of a selection line. A similar, but less extensive, experiment has reported similar patterns of variation in direct and correlated responses to selection in lines of *E. coli* that were selected on a range of carbon compounds (Fong et al., 2003).

In agreement with comparative studies, these experiments demonstrate that the evolution of adaptive phenotypic traits is broadly repeatable. Divergence in correlated responses to selection is typically extensive, implying that the genetic basis of these phenotypes vary widely among replicate radiations, suggesting that each instance of adaptive radiation leads to the evolution of a genetically unique assemblage of specialists that share similar adaptive traits.

**Constraints on adaptive radiation**

At some point, all adaptive radiations must come to an end. What factors do set the upper limit on the diversity that can evolve in any given radiation? This fascinating question has been the subject of several recent experiments that have investigated constraints on adaptive radiation using the familiar *P. fluorescens* microcosm system. Kassen et al. (2004) found that diversification of *Pseudomonas* into morph variant specialists peaked at intermediate levels of productivity and disturbance in unshaken microcosms. By culturing ancestral morph and derived morph genotypes separately and in competition, it was possible to demonstrate that reduced diversification at extremes of productivity and disturbance was attributable to a decrease in the competitive fitness of the derived WS morph under these conditions. In this system, ecological conditions alter the magnitude of the pleiotropic cost associated with resource specialization, constraining extensive diversification to environments of intermediate productivity and disturbance where the benefits of specialization exceed its costs. Genetic constraints may also limit adaptive radiation if all genotypes are not equally capable of specializing on the numerous resources that exist in heterogeneous environments. Buckling et al. (2003) have argued that generalists are likely to diversify rapidly, because mutations of small effect in a generalist genome will create at least rudimentary specialists phenotypes, while specialists are unlikely to diversify, because large mutations will be required to generate a phenotype specialized on a different resource. To test this hypothesis, Buckling and colleagues investigated the ability of derived genotypes specialized on the broth phase of the microcosm to diversify in microcosms lacking competitors. Specialist genotypes diversified into morph variants at a much slower rate than the broth-colonizing generalist ancestral genotype, directly demonstrating that specialists may be unable to diversify as a result of genetic constraints, even when the ecological opportunity for diversification exists.

While these studies have demonstrated that both ecological and genetic factors constrain diversification in microbial microcosms, this topic deserves a great deal more experimental investigation. Theory predicts that the productivity of different resources may impose an important constraint on diversification, because selection for specialization on resources that make only a minor contribution to total reproduction is weak (Kawecki, 1995; Holt, 1996). The rate of migration between patches containing different resources may also constrain the extent of diversification in an adaptive radiation, because the benefits of specialization are greater when migration between patches is low (reviewed in Felsenstein, 1976). Although experiments with *E. coli* and *Pseudomonas* illustrate that diversification is possible even when resources are perfectly mixed, it would be quite feasible to experimentally test the hypothesis that low migration between resource patches facilitates diversification into resource specialists.

**Discussion**

The important conclusions of the microbial literature on resource specialization and adaptive radiation can be summarized as follows. Resource heterogeneity generates divergent selection for specialization on alternative resources, irrespective of the spatial structure of the...
environment. If resource availability is spatially structured, divergent selection stems from differences in the resources available among patches; if resources are uniformly distributed, resource competition generates divergent selection within patches. Radiation is possible because trade-offs exist in the ability to exploit different resources, but these trade-offs are often not the result of antagonistic pleiotropy. At a genetic level, resource specialization is primarily the result of mutations that de-regulate gene expression. Finally, the overall tendency for similar adaptive radiations to repeat themselves at the ecological level is not reflected at the genetic level; similar adaptive radiations result from different genetic changes. In the following discussion, I discuss the implication of these findings for evolutionary theory, highlight some areas of future research, and briefly discuss some limitations of the use of microbial microcosms as model systems for adaptive radiation.

The notion that resource competition can ultimately result in sympatric speciation in sexual organisms remains controversial. Skepticism towards this hypothesis stems from both the difficulty of population genetic models to provide a realistic account of sympatric speciation (Dieckmann & Doebeli, 1999; Kondrashov & Kondrashov, 1999) and from the lack of field experiments that directly address the role of resource competition in evolutionary diversification (Bolnick, 2004; Schluter, 1994). Research on sympatric diversification in populations of microbes has focused on either the evolution of spatial specialization in *Pseudomonas* or the evolution of metabolic specialization in *E. coli*. These experiments show that diversification stemming from resource competition occurs with great ease in large populations that lack genetic exchange and recombination. Is this phenomenon a general feature of microbial populations, or does some peculiar feature of these organisms predispose them towards diversification?

Several lines of reasoning lead me to suspect that many adaptive radiations in selection experiments have gone unnoticed. A number of studies have reported the existence of genetic diversity within selection lines, but have not examined the ecological significance of this diversity (Adams & Oeller, 1986; Finkel & Kolter, 1999; Notley-McRobb & Ferenci, 1999a,b). We cannot conclude that this diversity reflects adaptive radiation, because genetic diversity can be transiently maintained in a microbial population subject to directional selection as a result of competition among ecologically equivalent lineages carrying independent beneficial mutations (Gerrish & Lenski, 1998). A more serious concern is that diversification has initially been recognized by colony morph variation in almost every published example of sympatric radiation (Helling et al., 1987; Korona et al., 1994; Rainey & Travisano, 1998; Rozen & Lenski, 2000; Friesen et al., 2004). Relying on colony morph diversity as a surrogate of genetic diversity imposes an important bias against the detection of adaptive radiation in selection experiments, because microbiologists have long recognized that much of microbial diversity is not reflected in colony morph variation.

How can we overcome this limitation? Early advances in microbial taxonomy relied on the use of biochemical tests to identify variation among bacteria. For example, the enormous diversity of *Pseudomonas* was not fully appreciated or understood until biochemical tests were used to classify a wide variety of *Pseudomonas* strains (Stanier et al., 1966). The current classification of *Pseudomonas* remains largely unchanged, revealing the potential power of biochemical tests to catalogue microbial diversity (Spiers et al., 2000). Phenotypic microarrays (Bochner, 2003) now allow researchers to rapidly score thousands of biochemical characters of bacterial genotypes and communities, potentially providing a powerful tool to search for diversity in microbial microcosms. Genetic markers provide an alternative method to identify adaptive radiations in microbial populations. This approach takes advantage of the fact that beneficial mutations are completely linked to marker loci in nonrecombining bacterial genomes. The rise of a novel genotype will, therefore, cause a shift in the frequency of a given neutral marker. If multiple markers are maintained in evolving populations, one can infer that genetic diversity exists in the population, even if it is not reflected in colony morphology. The use of this approach allowed the identification of a ‘cryptic’ adaptive radiation in *Pseudomonas* that did not involve the emergence of colony morph variants (MacLean et al., 2005). However, two serious limitations are associated with this approach. The first of these is that multiple genotypes can evolve within the same marker background. Secondly, genetic markers that are eliminated from evolving populations are lost forever. Both of these difficulties can be overcome to some extent by using genotypes that rapidly generate neutral genetic variation at a marker locus (e.g. Imhof & Schlotterer, 2001).

If we accept that sympatric diversification is likely a general feature of microbial evolution in heterogeneous environments, how can this research programme be extended? Microbial selection experiments have successfully investigated the causes of diversification, but patterns of diversification in microcosms remain poorly understood. For example, it is clear that diversity can be maintained in microbial microcosms for hundreds (Rainey & Travisano, 1998; MacLean et al., 2005) or thousands (Helling et al., 1987; Rozen & Lenski, 2000) of generations, but it is unclear if this reflects the persistence of specialist lineages that evolve early in the history of selection experiments or the continual diversification of generalists into specialist lineages (Rozen & Lenski, 2000). Addressing this question will require a detailed knowledge of the phylogeny of selection lines. At present, such information is not available because most selection experiments do not use variable genetic markers within lines, and because sequence diversity in
conserved genes that are typically used to reconstruct phylogenies evolves at too slow a rate to be of any use in reconstructing the phylogeny of experimental populations of microbes (Lenski et al., 2003).

A second avenue for further research that may yield interesting results concerns the co-evolution of ecological interactions during adaptive radiation. A general feature of microbial adaptive radiations is that fitness in communities assembled by adaptive radiation is frequency dependent, implying that the fitness of any given genotype is determined to a large extent by the ecological interactions of that genotype with other genotypes. Ecological interactions among microbial genotypes may be complex and may involve both competition for shared resources and the release of metabolic by-products that may directly promote or inhibit the growth of other genotypes (Rosenzweig et al., 1994; Rozen & Lenski, 2000; Hodgson et al., 2002; Kerr et al., 2002; Rainey & Rainey, 2003). Theoretical models predict that interactions themselves ought to evolve (Taper & Case, 1992; Doebeli & Dieckmann, 2000; Doebeli, 2002), but selection experiments have not tracked how interactions change during the course of adaptive radiation, let alone explicitly tested theoretical predictions about how interactions ought to evolve.

The use of microbial microcosms as model systems in evolutionary ecology remains controversial (Jessup et al., 2004). Many evolutionary ecologists are skeptical of the utility of microbial selection experiments because they involve the use of simple organisms and environments. A full reply to these criticisms is beyond the scope of this manuscript, so I will discuss two very general limitations of the experiments reviewed in this paper that may bias the outcome of these experiments.

Selection experiments with asexual microbes are limited by the fact that they cannot provide an account of speciation, which requires the evolution of reproductive isolation among sexual lineages. Although asexual genotypes and sexual species are formally equivalent, in that they constitute discrete genetic entities, the process by which new asexual genotypes arise, spontaneous mutation, is generally discounted as a general explanation for the origin of sexual species. However, selection experiments with asexual microbes provide a rigorous account of the ecological causes of divergent selection, which is a central feature of most theories of sympatric and allopatric speciation in sexual organisms (reviewed in Schluter, 2001). One of the interesting current challenges to experimental microbial ecologists is to apply the approaches that have been used to study adaptive radiation in asexual microbes such as E.coli and Pseudomonas to sexual microorganisms such as S. cerevisiae and Chlamydomonas. Such experiments could provide much needed empirical tests of existing theories of speciation.

All of the experiments reviewed in this manuscript studied adaptation and diversification in simple communities that lack trophic structure. It might be argued that selection for resource specialization is likely to be much weaker in natural communities, because exploitation by predators and parasites can have a profound impact on the strength of resource competition (reviewed in Holt & Lawton, 1994). Trophic structure can be easily added to bacterial microcosms through the introduction of virulent bacteriophage, voracious predators of bacteria (Lenski & Levin, 1985; see reviews in Bohannan & Lenski, 2000). The general conclusion of these studies is that predation alters the strength of divergent selection stemming from resource competition, suggesting that the outcome of many of the experiments reviewed in this paper might have been different in the presence of predators. For example, Buckling & Rainey (2002) found that exploitation by phage greatly reduced the extent of diversification of Pseudomonas into morph variants within a given microcosm, apparently as a result of relaxed resource competition stemming from the ≈4-fold reduction in bacterial density in microcosms containing phage. In spatially unstructured microcosms that offer minimal opportunity for diversification into morph variants in the absence of predation, exploitation by phage substantially increased morph diversification as a result of the appearance of phage-resistant morph variants that would normally be competitively excluded from unstructured microcosms as a result of their poor ability to compete for resources (Brockhurst et al., 2004). These simple experiments illustrate that increasing the biological complexity of the arena in which selection takes place can change the outcome of selection. Note, however, that in both of these cases, diversification is still driven by resource competition.

The scientific method is most powerful when we are able to study the individual components of complex processes in isolation. Microbial selection experiments offer researchers the opportunity to do exactly this, albeit in an arbitrary and highly artificial setting. The limitation of this research programme is that it does not provide any information regarding the relative importance of these mechanisms outside of the lab. Despite this caveat, it is tempting to speculate that patterns of diversification in nature reflect the operation of the processes that laboratory populations of microorganisms illustrate so clearly.

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