

LETTER

Resource competition and adaptive radiation in a microbial microcosm

R. Craig MacLean,^{1*} Alison Dickson² and Graham Bell¹

¹Department of Biology, McGill University, 1205 Ave.

Dr. Penfield, Montreal

H3A 1B1, QC, Canada

²Department of Pharmacology and Therapeutics, McGill

University, 3655 Promenade

Sir-William-Osler, Montreal

H3G 1Y6, QC, Canada

*Corresponding: E-mail:

c.maclea@ic.ac.uk

Abstract

Theory predicts that competition for shared resources in a monomorphic population generates divergent selection for adaptation to alternative resources. Experimental tests of this hypothesis are scarce. We selected populations of the bacterium *Pseudomonas fluorescens* in spatially homogeneous microcosms containing a complex mixture of resources. Initially, all populations consisted of two isogenic clones. The outcome of selection was the evolution of a diverse community of genotypes within each population. Sympatric genotypes exhibited differentiation in metabolic traits related to resource acquisition and frequency-dependent trade-offs in competitive ability, as we would expect if different genotypes consumed different resources. These results are consistent with the hypothesis of adaptive radiation driven by resource competition. Reconciling the results of this study with those of earlier experiments provides a new interpretation of the ecological causes of adaptive radiation in microbial microcosms.

Keywords

Adaptive radiation, Biolog, character displacement, competition, experimental evolution, generalists, *Pseudomonas*, specialists, sympatric speciation, trade-off.

Ecology Letters (2005) 8: 38–46

INTRODUCTION

Understanding the causes of adaptive radiation is an outstanding problem in evolutionary biology. It has often been argued that adaptive radiation is the ultimate result of divergent natural selection stemming from differences in the available resources *among habitats* and from competition for common resources *within the same habitat* (reviewed in Schluter 2000b). The underlying premise of models that examine the evolutionary consequences of resource competition in sympatry is that intense competition for commonly exploited resources generates divergent selection for specialization on underutilized, or novel, resources. Adaptive radiation of an initially monomorphic population into specialist lineages is a robust outcome of these models in asexual organisms (Geritz *et al.* 1998; Doebeli & Dieckmann 2000), provided that the environment is characterized by a diversity of resources (for example, a normal distribution of prey size) and that trade-offs exist in the ability to exploit alternative resources (for example, small vs. large prey). In sexual organisms, the same ecological mechanisms generate divergent selection (Slatkin 1980; Doebeli 1996), but adaptive radiation of a single lineage into distinct specialist species will only occur if

assortative mating evolves within ecologically divergent subpopulations (Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999). Despite the existence of a rich body of theory, direct experimental tests of the role of resource competition in adaptive radiation in natural populations remain limited (Schluter 1994, 1995; Bolnick 2004).

In an attempt to circumvent the difficulties associated with performing rigorous and replicated experiments in the field, a number of researchers have started to investigate the ecological causes of adaptive radiation in microbial microcosms where both organisms and environments can be manipulated with ease (see reviews in Rainey *et al.* 2000; Travisano & Rainey 2000). A critical test of the role of resource competition in adaptive radiation was provided by Rainey & Travisano (1998), who selected a single clone of the bacterium *Pseudomonas fluorescens* in microcosms containing a complex mixture of resources that were incubated without physical disturbance (spatially structured microcosms) or with continuous shaking (unstructured microcosms). The ancestral SM (smooth) morph genotype, which colonized the broth of the microcosm, diversified into a range of genetically differentiated specialist morph variants in spatially structured microcosms. The most prominent morphs were wrinkly-spreader (WS), which colonized the

air-broth interface of microcosms, fuzzy-spreader (FS), which colonized the bottom of microcosms, and SM, which continued to colonize the broth of the microcosm. The ancestral genotype did not diversify into morph variants in shaken microcosms, presumably because these lack spatially distinct niches of that can support populations of specialist morphs variants of *Pseudomonas*. This system has since become an important microbial system for studying the ecology (Travisano & Rainey 2000; Buckling & Rainey 2002; Brockhurst *et al.* 2004) and genetics (Spiers *et al.* 2002; Buckling *et al.* 2003; Spiers *et al.* 2003; MacLean *et al.* 2004) of adaptive radiation.

The results of Rainey & Travisano (1998) suggest that spatial heterogeneity in resource availability is required for adaptive radiation, despite the existence of diverse resources in unstructured microcosms. This result appears to contradict the large body of theory on resource competition and adaptive radiation. Here we re-examine the outcome of natural selection in unstructured microcosms. To provide simple insights into the dynamics of selection in unstructured microcosms, we initiated our selection lines with two genotypes of *Pseudomonas* that carry different genetic markers. This technique demonstrated that a diverse assemblage of broth-colonizing genotypes evolved within each selection line. Independently evolved sympatric genotypes were then isolated from each selection line to test two important predictions of the theory of adaptive radiation stemming from resource competition (Schluter 1995, 2000a; Rainey & Travisano 1998): (1) adaptive radiation stemming from resource competition results in the evolution of communities consisting of genotypes that consume different resources; (2) trade-offs in competitive ability among resource specialists maintain diversity in communities assembled by adaptive radiation stemming from resource competition. Our results support both of these important predictions and allow several alternative ecological scenarios for adaptive radiation to be ruled out.

MATERIALS AND METHODS

Selection experiment

We inoculated 10 25 mL glass vials containing 6 mL of M9KB (glycerol, 10 g L⁻¹; proteose peptone no. 3, 20 g L⁻¹; Na₂HPO₄, 6 g L⁻¹; KH₂PO₄, 3 g L⁻¹; NH₄Cl, 1 g L⁻¹; NaCl, 0.5 g L⁻¹; pantothenate, 0.0024 % (w:v) with a mixture of *Pseudomonas fluorescens* SM morph genotypes SBW25 and SBW25 *panB*⁻, an isogenic mutant containing a complete deletion of the *panB* gene, which is required for the biosynthesis of the vitamin pantothenate. Vials were incubated at 28 °C with constant, vigorous, shaking (200 rpm). Selection lines were thoroughly vortexed and diluted 100-fold into fresh culture medium every day for 24

transfers. The minimum population size of each selection line at the bottleneck imposed by this transfer regime was $c. 1 \times 10^7$. At each transfer, a sample of each microcosm was diluted and spread on indicator plates containing a low concentration of pantothenate (glycerol, 5 g L⁻¹; casamino acids, 10 g L⁻¹; Na₂HPO₄, 6 g L⁻¹; KH₂PO₄, 3 g L⁻¹; NH₄Cl, 1 g L⁻¹; NaCl, 0.5 g L⁻¹; pantothenate 2.4 × 10⁻⁶%) that allows pan⁻ and pan⁺ genotypes to be easily distinguished based on colony size. We scored the morphology and marker of a large number of colonies from each line at each transfer (median = 361, upper quartile = 467, lower quartile = 207). At the end of the experiment, all 10 selection lines were frozen at -80 in 50% glycerol (wt : vol) and two randomly chosen SM genotypes with opposing markers were isolated from each line for further assays.

Phenotypic assays

To assay for metabolic differentiation within selection lines, we assayed evolved and ancestral SM genotypes on Biolog GN2 microplates (Biolog, Hayward, CA, USA). Biolog plates are 96 well microtitre plates in which all wells contain an indicator dye of carbon metabolism as well as a simple growth medium. Ninety-five wells contain a unique carbon compound that acts as the sole available carbon source in the well and the remaining well is a carbon-free control. To assay a given genotype on a Biolog plate, we first grew a single colony overnight in M9KB supplemented with pantothenate (28 °C, 200 rpm). 20 µL of this culture was then diluted in 20 mL of M9 salts supplemented with excess pantothenate (Na₂HPO₄, 6 g L⁻¹; KH₂PO₄, 3 g L⁻¹; NH₄Cl, 1 g L⁻¹; NaCl, 0.5 g L⁻¹ pantothenate 0.0024% (w:v) and incubated for $c. 2$ h at 28 °C to starve the cells. 150 µL of starved cells was then added to each well on the microplate, which was read at 660 nm using an automated microplate reader (Bio-tek Elx800-NB) after 24 h of incubation ±5 min. We calculated a quantitative score for each well (A_{660} corr.) as the observed absorbance minus the blank control. Under these conditions, A_{660} corr. values are well correlated with cell densities ($r^2 = 0.76$) and no growth occurs in the carbon-free control well. The ancestral genotypes used in this experiment consistently produce growth on 48 of the 95 substrates after 24 h of incubation (i.e. A_{660} corr. > 0.2). On these substrates, little variation exists among independent replicates of the ancestral clones. Evolved genotypes seldom produce detectable growth on any of the remaining 47 substrates, so we only used data from the 48 ancestral substrates in our analysis. Euclidean distances were calculated between all possible pairs of genotypes by treating A_{660} on each substrate as a single character, as previously described (MacLean & Bell 2003). Clustering was performed using a UPGMA algorithm.

Growth in M9KB

To determine if metabolic variation reflects differences in the ability to grow in microcosms, we assayed the ability of evolved and ancestral genotypes to grow independently in M9KB. To assay a given genotype in M9KB, we first grew up an overnight culture of the genotype in a vial containing 5 mL of pantothenate supplemented M9KB that was incubated at 28 °C with vigorous shaking (200 rpm). 100 µL of this pre-innoculation culture were then diluted down by 100X into pantothenate supplemented M9KB. 200 µL of this culture was then added to into eight different wells on a 96-well microtiter plate. This microplate was incubated at 28 °C. The absorbance of this plate was scored at \approx 1 h intervals over an 8 h period. Growth rate was estimated as the slope of the regression of absorbance on time over the interval of time when population growth rate is approximately linear. After 24 h of incubation, 40 µL was sampled from each well and added to 180 µL of M9 salts on a fresh microplate. The absorbance of this plate was measured at 660 nm. Cell titres were estimated using a standard curve of absorbance on cell titre.

Fitness assays

To assay for ecological differentiation, we directly competed oppositely-marked SM morph genotypes against each other. Prior to competition, each genotype was first grown overnight to stationary density in M9KB supplemented with excess pantothenate. Pre-inoculation cultures of oppositely marked genotypes were then mixed together and inoculated into duplicate vials where they were

allowed to compete overnight under the culture conditions used in the selection experiment. Initial and final ratios of the two competing genotypes were determined by plating on indicator plates. Competitive ability was calculated as $\log w$, the change in log ratio of competitors (Hartl & Clark 1997), such that a value of 0 indicates equal competitive ability.

RESULTS

Marker dynamics

At the start of the selection experiment, all of our populations consisted of a \approx 1 : 1 mixture of isogenic SM-morph genotypes carrying the pan+ or pan- marker. If selection in this system is directional, the ancestral genotypes would eventually be replaced by a mutant with greater overall competitive ability, resulting in the fixation of one of the two genetic markers in each selection line through genetic hitch-hiking. We did not observe such selective sweeps in any line. Rather, SM genotypes carrying both genetic markers persisted in all 10 selection lines, despite large fluctuations in marker frequency through time. Moreover, we found that SM morph genotypes co-existed with a morphological variant resembling WS that evolved in all 10 selection lines during the selection experiment. The neutral marker (pan+ or pan-) of these genotypes was difficult to determine without laborious replicate plating due to the large variance in colony size among WS genotypes with the same neutral marker. Given that this morph variant was both conspicuous and heritable, we treated these genotypes as a third marker class (Fig. 1).

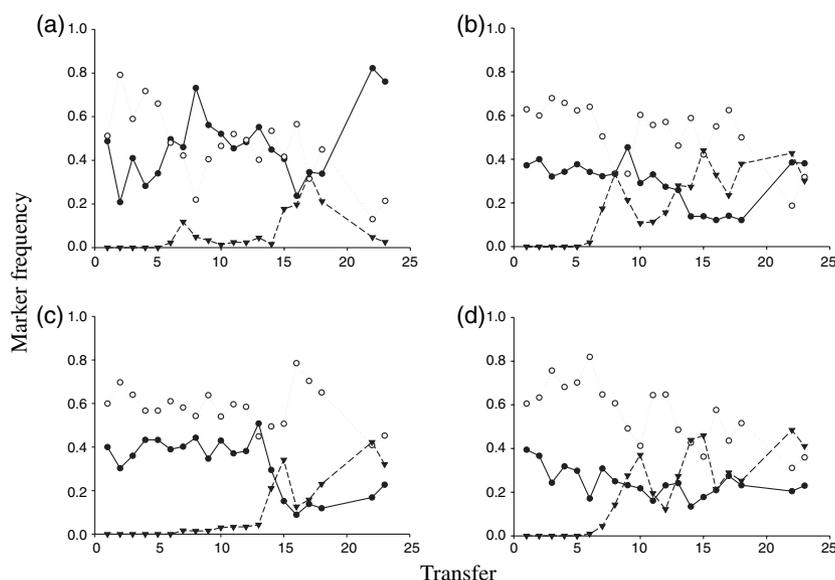


Figure 1 Dynamics of genetic markers in selection lines. Each graph (a–d) shows the dynamics of the three genetic markers in an independent selection line. Initially, all lines consisted of SM morph genotypes carrying the pan+ (open circle) or pan- (filled circle) marker. During the course of the experiment, colony morph variants evolved, providing a third genetic marker (triangle).

Metabolic differentiation among sympatric genotypes

When re-inoculated into shaken microcosms, morph variant genotypes colonize the walls of the microcosm, while evolved SM genotypes colonize the broth of the microcosms. If the co-existence of oppositely-marked SM morph genotypes within selection lines reflects adaptive radiation driven by competition for resources, sympatric pairs of SM genotypes should exhibit differentiation in phenotypic characters related to resource acquisition (Schluter 2000b). To test this prediction, we assayed the ability of SM morph genotypes to consume a large range of carbon sources provided by Biolog GN2 microplates. Growth on different carbon sources reflect the activity of different metabolic pathways (Bochner 2003), allowing cryptic metabolic variation among genotypes to be detected (for examples Riley *et al.* 2001; MacLean *et al.* 2004).

Multivariate clustering algorithms group SM morph genotypes into two principle clusters based on catabolic profile (Fig. 2). The difference between these cluster primarily reflects quantitative differences in the extent of growth on single carbon substrates by genotypes from different clusters, and not qualitative differences in the substrates that can be used to support growth by genotypes from different clusters. To characterize the metabolic traits associated with each cluster, we fitted growth data to a model that included terms for cluster, substrate, and cluster \times substrate interaction (Table 1). This model attributes almost all of the variation to the main effects of cluster and substrate. A statistically significant, but very minor, fraction of the variance is attributable to the interaction between cluster and substrate, apparently because cluster A genotypes have superior growth on all the carbon substrates

present on Biolog plates. In each selection line, one genotype belongs to each cluster. Although both the pan- and pan+ ancestral genotypes are members of cluster A, only pan+ genotypes from selection lines belong to this cluster. The probability of observing this surprising result under the null hypothesis that the pan marker has no effect on the evolution of SM metabolic profile is $(0.5^2 + 0.5^2)^{10} = 1 \times 10^{-4}$.

The ecological consequences of metabolic variation

What are the ecological consequences of the metabolic variation among sympatric genotypes? To address this question, we measured the stationary density and growth rate of genotypes from both metabolic clusters in the complex culture medium used in our microcosms, M9KB. The pan- and pan+ ancestral genotypes have indistinguishable stationary densities ($t_{14} = 1.1$, $P = 0.29$) and growth rates ($t_{14} = 1.0$, $P = 0.32$) in M9KB. Stationary density and growth rate are positively correlated among genotypes from selection lines ($F_{1,8} = 15$, $P = 0.005$, $r^2 = 0.66$), so we chose to focus on stationary density as a surrogate of growth in M9KB. Metabolic ability and growth in M9KB are positively correlated among genotypes from selection lines (Fig. 3: $F_{1,8} = 28$, $P = 0.0007$, $r^2 = 0.78$), directly demonstrating that variation in metabolic traits is related to variation in the ability to consume the ensemble of resources present in our culture medium.

Competition among sympatric genotypes

The results of our metabolic and growth assays illustrate that sympatric pairs of genotypes are differentiated with respect to

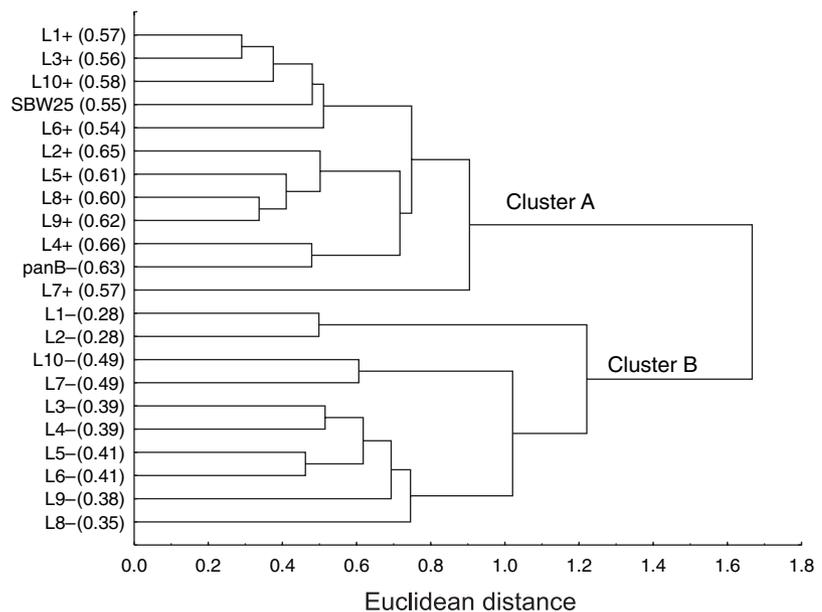


Figure 2 Metabolic differentiation among evolved genotypes. Metabolic profile was quantified by measuring absorbance on the 48 informative substrates provided by Biolog microplates. Tips are designated according to the selection line of origin (1–10) and neutral marker (+ or –) of each genotype; SBW25 and panB– are the two ancestral genotypes. Numbers in brackets denote the average absorbance score of each genotype over all substrates. Clusters correspond to genotypes with broad catabolic function resembling the ancestral clone (cluster A) or genotypes with narrow catabolic function (cluster B).

Effect	d.f.	SS	MS	<i>F</i>	<i>P</i> -value	% Total variance explained
Cluster	1	10.4	10.4	346	< 0.0001	28.2
Substrate	47	43.5	0.93	116	< 0.0001	58.7
Cluster × substrate	47	1.34	0.029	3.62	< 0.0001	2.7
Error	864	6.83	0.008			10.3

Table shows the results of a mixed-model ANOVA performed on the Biolog performance of evolved genotypes (Fig. 2). Cluster (A or B) was treated as fixed factor and substrate was treated as a random factor. Changing substrate to a fixed factor has little effect on the outcome of this analysis.

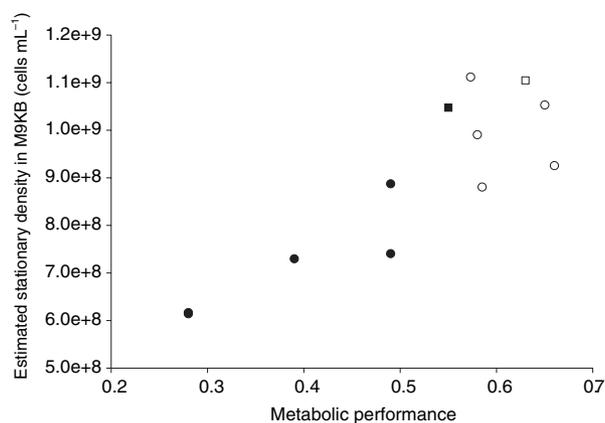


Figure 3 Metabolic performance and growth in M9KB. Plotted points show the estimated stationary density in M9KB and metabolic performance of genotypes from selection lines (circles) and the ancestral genotypes (squares). Open symbols designate pan⁺ genotypes and closed symbols designate pan⁻ genotypes. Metabolic performance was calculated as the average absorbance score of each genotype across all 48 Biolog substrates. Stationary density was estimated by fitting absorbance data from eight independent M9KB cultures of each genotype to a standard curve of cell density and absorbance.

phenotypic traits that are involved in resource exploitation. Genotypes specialized on alternative resources should express negative frequency-dependent trade-offs in competitive ability. To directly test for trade-offs, we competed sympatric pairs of metabolic variant genotypes against each other across a wide range of initial frequency of each of the two competing genotypes. As a control for the effect of the pan marker on competitive ability, we also competed the pan⁻ and pan⁺ ancestral genotypes against each other. The competitive ability of the ancestral genotypes is independent of initial frequency ($F_{1,3} = 0.59$, $P = 0.52$), and no difference exists in the overall mean competitive ability of the two ancestral genotypes ($t_{11} = 1.12$, $P = 0.28$). Genotypes from cluster A and B tend to enjoy a competitive advantage in a microcosm dominated by the opposing type (Table 2). Note that genotypes from cluster B enjoy a larger competitive advantage when initially rare than do genotypes from cluster

Table 1 Metabolic profile of specialist and generalist genotypes

Table 2 Competition among metabolically differentiated genotypes

Selection line	Competitive ability of cluster B genotype when rare	Competitive ability of cluster A genotype when rare	Rate of decline in competitive ability	r^2
1	1.62 (0.30)*	1.41 (0.21)*	-3.0 (0.47)*	0.96
2	0.72 (0.31)	2.23 (0.50)*	-2.9 (0.65)*	0.91
3	1.91 (0.20)**	1.08 (0.15)*	-3.0 (0.32)**	0.97
4	1.97 (0.13)**	0.42 (0.25)	-2.39 (0.35)**	0.96
5	-0.64 (0.42)	1.3 (0.58)	-0.66 (0.87)*	0.23
6	0.74 (0.23)*	1.0 (0.18)*	-1.7 (0.37)*	0.92
7	0.52 (0.097)*	0.12 (0.11)	-0.65 (0.18)*	0.87
8	-0.86 (0.35)	1.0 (0.33)*	-0.13 (0.60)	0.03
9	0.41 (0.94)	1.3 (1.4)	-1.7 (2.2)	0.40
10	0.07 (0.10)	0.59 (0.10)*	-0.67 (0.18)*	0.86
Mean	0.64 (0.31)*	1.05 (0.18)***	-1.7 (0.33)***	

The table shows the outcome of competitions between pairs of genotypes from each of the 10 selection lines. Competitions were carried out across four initial ratios of the two competing genotypes, ranging from 10 to 90%. Competitive ability was estimated as $\log w$, the change in the log of the ratio of competing genotypes, such that a value of $\log w = 0$ indicates equal competitive ability. Competitive ability when rare was estimated as the y -intercept of a linear regression of competitive ability on initial competitor frequency and the rate of decline in competitive ability was calculated as the slope of this regression. Rate of decline in competitive ability provides an estimate of difference in the fitness of a genotype when it is initially rare (initial frequency ≈ 0) and when it is dominant (initial frequency ≈ 1). If the value of this slope is less than zero, the fitness of the genotype is negative frequency-dependent. Values in brackets denote the standard error associated with each estimate; r^2 is the proportion of variance in competitive ability attributable to initial frequency. The statistical significance of each estimate was calculated using one-tailed t -tests with an appropriate degree of freedom * $0.05 < P < 0.01$; ** $0.01 < P < 0.001$; *** $P < 0.001$.

A, although this difference is not statistically significant ($F_{1,18} = 1.24$, $P = 0.24$). In every case, relative competitive ability is negative frequency-dependent (Table 2).

DISCUSSION

Summary

Our experiment introduced two isogenic clones of *Pseudomonas* into a novel environment containing a diversity of resources in the form of multiple carbon substrates. The response to selection in this experiment was the evolution of a diverse community of broth and rim colonizing genotypes within each selection line (Fig. 1). The theory that we wish to test is explicitly concerned with the ecological causes of adaptive radiation in complete sympatry, so we chose to focus our assays on understanding the mechanisms of diversification within the broth-colonizing population of each selection line. Sympatric adaptive radiation driven by resource competition is expected to result in the evolution of a diverse community of genotypes that are ecologically differentiated as a result of adaptation to different resources. In support of this prediction, diversification into metabolic variants that differ in their ability to exploit the resources in the broth occurred within each selection line (Fig. 2 and 3). Adaptive radiation stemming from resource competition is only possible if genetic trade-offs exist in the ability to exploit different resources. Direct evidence for the existence of such trade-offs comes from the finding that competitive fitness is negative frequency-dependent among metabolic variants isolated from all 10 lines (Table 2). In eight of 10 lines, metabolic variants were mutually invisable, such that either metabolic variant was able to invade a population dominated by the alternative variant. Mutual invisibility implies that the magnitude of the trade-off in competitive ability between metabolic variants was sufficient to maintain the co-existence of metabolic variants in the broth. Although these results are consistent with the hypothesis of adaptive radiation stemming from resource competition for the diverse carbon substrates in the broth, some features of our results deserve further comment and alternative ecological causes of adaptive radiation must be addressed.

Marker effects

At the outset of the experiment, each selection line contained a base population consisting of an isogenic mixture of pan⁺ and pan⁻ ancestral genotypes of *Pseudomonas*. The pan⁻ ancestor is unable to synthesize the essential vitamin pantothenate and cannot grow unless provided with an exogenous source of pantothenate. We deliberately added a large excess of pantothenate to the culture medium in an attempt to compensate for the effects of the *panB* deletion. Under the conditions of our experiment, the ancestral pan⁻ and pan⁺ genotypes have equal growth rates and competitive ability, directly demonstrating that our experimental protocol compensated for any fitness effects of the pan marker. Despite the

apparent neutrality of the pan marker in the base population, the marker clearly pre-disposes pan⁻ genotypes towards the evolution of impaired metabolism (Fig. 2). This frustrating, albeit interesting, result complicates the interpretation of our results, because it is somewhat unclear if metabolic differences between sympatric genotypes (Fig. 2) reflect adaptation to different resources or the long-term consequences of variation at the pan marker locus. In the most extreme case, it might be argued that the use of a variable base population was the immediate cause of metabolic diversification within each line, because impaired metabolism always evolves in a pan⁻ background and pan⁺ genotypes are incapable of evolving impaired metabolism.

The most straightforward way to evaluate the evolutionary consequences of the *panB* deletion would be to repeat our experiment by initiating each selection line with either a pan⁺ or pan⁻ genotype and to test for adaptive radiation using the method described in this manuscript. Unfortunately, such an experiment would be faced with two important technical difficulties. Detecting adaptive radiation within lines would require regular and intensive assays of the metabolic variation present within each line because of the lack of conspicuous morphological differences between metabolic variants. Performing competitions between sympatric pairs of genotypes would also be laborious, because a suitable neutral marker that allows colonies of different genotypes to be distinguished on agar plates would have had to have been introduced into at least one genotype from each selection line. In summary, we argue that the use of a genetically variable base population was a necessary feature of the design of this experiment, without which we would have almost certainly failed to detect adaptive radiation within the SM population.

Our approach to further investigate the consequences of the *panB* deletion for metabolic evolution has been to conduct intensive assays of the metabolic variation within two focal lines (R.C. MacLean *et al.*, unpublished). These assays have revealed the existence of pan⁺ genotypes with impaired metabolism (i.e. Fig. 2, members of Cluster B) and pan⁻ genotypes with unimpaired metabolism (i.e. Fig. 2, members of cluster A), implying that metabolic diversification within lines is not purely attributable to variation at the pan marker locus. This important result suggests that the outcome of selection would have been adaptive radiation into metabolic variants had we initiated each selection with either a pan⁺ or pan⁻ ancestor. The evolutionary bias imposed by the genetic marker, we employed illustrates that seemingly neutral genetic variation can have important evolutionary consequences over hundreds of generations of selection. Unfortunately, this bias complicates the interpretation of our results regarding the evolutionary causes of metabolic diversification.

The costs and benefits of impaired metabolism

If we accept that impaired metabolism is not simply an artefact of the use of the pan– marker, how can we account for the evolution of this phenotype? The frequency-dependent trade-off in competitive ability between metabolic variants genotypes (Table 2) implies that impaired metabolism is associated with both costs and benefits. The inability of genotypes with impaired metabolism to effectively exploit the ensemble of resources in the broth (Fig. 3) provides a simple explanation for why this phenotype is associated with a cost. According to the hypothesis of adaptive radiation stemming from resource competition, the benefit of impaired metabolism must reflect the ability of genotypes with impaired metabolism to effectively exploit a subset of the resources in the broth. However, assays of metabolic performance fail to detect specialization on individual carbon substrates by genotypes with impaired metabolism (Table 1). One possible explanation for this discrepancy is that our metabolic assay did not detect the benefits associated with catabolic specialization, simply because we did not assay growth on the appropriate carbon sources. In support of this argument, many of the substrates present in M9KB (especially peptides) are not included on the Biolog plates that we used to assay metabolic profiles; conversely, many of the substrates present on Biolog plates are not present at high concentrations in M9KB. Determining the precise ecological consequences of metabolic variation would have been greatly simplified had we selected *Pseudomonas* in a complex environment containing a well-defined mixture of carbon substrates. We will report the result of such experiments in a forthcoming paper.

Alternatives to competition

It is important to emphasize that a range of ecological mechanisms other than resource competition can cause adaptive radiation in microbes (Levin 1988; Doebeli & Dieckmann 2000; Rainey *et al.* 2000; Travisano & Rainey 2000). In particular, it has been argued that temporal fluctuations in resource availability and cross-feeding interactions among genotypes can result in adaptive radiation. Both of these mechanisms invoke negative frequency-dependent selection as the evolutionary mechanism that maintains diversity in communities assembled by adaptive radiation, so that the existence of negative-frequency dependence cannot be used to discount alternatives to resource competition as the ecological mechanisms of divergence.

Stewart & Levin (1973) demonstrated mathematically that seasonal environments can generate divergent natural selection for specialization on low and high resource

concentrations, provided that a trade-off exists in the ability to exploit scarce and abundant resources. We found that early log-phase growth rate and stationary density are positively correlated in M9KB. These two measures of growth reflect the ability to consume abundant and scarce resources, respectively, providing strong evidence that temporal variation in resource availability was not the ecological cause of adaptive radiation in our experiment. Cross-feeding between genotypes has also been invoked as an ecological cause of adaptive radiation in microbial microcosms (Helling *et al.* 1987; Rosenzweig *et al.* 1994; Turner *et al.* 1996; Rozen & Lenski 2000; Porcher *et al.* 2001; Pfeiffer & Bonhoeffer 2004). In this scenario, adaptation to a novel culture medium initially occurs through directional selection for an increased rate of substrate assimilation. Genotypes with an increased rate of substrate assimilation secrete large quantities of secondary metabolites into the culture medium, providing the ecological opportunity for the evolution of specialization on either the exogenously supplied resource or secondary metabolites derived from these resources. In our experiment, selection lines were serially diluted into fresh culture medium by 100-fold on a daily basis. Daily dilution of culture medium is thought to prevent the evolution of cross-feeding by preventing the accumulation of secondary metabolites to high concentration (Doebeli 2002) and empirical data from selection experiments are in good agreement with this prediction (Rosenzweig *et al.* 1994; Treves *et al.* 1998; Rozen & Lenski 2000; Doebeli 2002). We conclude that neither seasonality or cross-feeding provide a satisfactory explanation to explain why adaptive radiation occurred in our selection experiment.

CONCLUSION

In simple environments, containing a single resource, the dynamics of microbial evolution are thought to be characterized by periodic selective sweeps that replace the dominant genotype in a population with a fitter descendant (Novick & Szilard 1950; Atwood *et al.* 1951; Crow & Kimura 1965). The population then remains essentially fixed for a single genotype until the next selective sweep. This model portrays evolution in asexual populations as a gradual progression from a genotype of sub-optimal fitness to a genotype of optimal fitness. A number of selection experiments have reported results that are consistent with this paradigm (Novick & Szilard 1950; Atwood *et al.* 1951; Paquin & Adams 1983; Lenski & Travisano 1994). In this paper, we investigate the influence of resource heterogeneity on the outcome of natural selection in bacterial populations. Although we took advantage of a model system developed by Rainey & Travisano (1998), it is important to point out that we did not replicate some precise details of their

experimental design. For example, selection lines in the experiment of Rainey and Travisano were not transferred to fresh culture medium on a daily basis. The profound differences in the chemical composition of shaken microcosms that are routinely transferred or allowed to degrade during a week long period of bacterial growth presumably explain why rim-colonizing WS genotypes only appeared in shaken microcosms in the present study. Despite these methodological differences, both studies demonstrate that the consequence of selection in complex environments is adaptive radiation into specialist lineages. In spatially structured microcosms, competition for resources that have a characteristic spatial distribution, such as oxygen, generates divergent selection for specialization on spatially distinct regions of the microcosm. In unstructured microcosms, diversification occurs as a result of competition for resources that are uniformly distributed throughout the microcosms, such as carbon substrates.

ACKNOWLEDGEMENTS

We would like to thank A. Buckling, S. Smith, R. Barrett, and S. Collins for insightful comments and discussion. Paul Rainey and two anonymous referees provided helpful suggestions and criticisms that improved an earlier version of this manuscript. Ancestral genotypes were a kind gift of P. Rainey. Funding was provided by grants from the Natural Sciences and Engineering Research Council of Canada to G.B., R.C.M., and A.D. Technical assistance was provided by M. Pearlstein.

REFERENCES

- Atwood K.C., Schneider L.K. & Ryan F.J. (1951). Periodic selection in *Escherichia coli*. *Proc. Natl Acad. Sci. U S A*, 37, 146–155.
- Bochner B.R. (2003). New technologies to assess genotype-phenotype relationships. *Nat. Rev. Genet.*, 4, 309–314.
- Bolnick D.I. (2004). Can intraspecific competition drive disruptive selection? An experimental test in natural populations of sticklebacks. *Evolution*, 58, 608–618.
- Brockhurst M.A., Rainey P.B. & Buckling A. (2004). The effect of spatial heterogeneity and parasites on the evolution of host diversity. *Proc. R. Soc. Lond. B*, 271, 107–111.
- Buckling A. & Rainey P.B. (2002). The role of parasites in sympatric and allopatric host diversification. *Nature*, 420, 496–499.
- Buckling A., Wills M.A. & Colegrave N. (2003). Adaptation limits diversification of experimental bacterial populations. *Science*, 302, 2107–2109.
- Crow J.F. & Kimura M. (1965). Evolution in sexual and asexual populations. *Am. Nat.*, 99, 439–450.
- Dieckmann U. & Doebeli M. (1999). On the origin of species by sympatric speciation. *Nature*, 400, 354–357.
- Doebeli M. (1996). An explicit genetic model for ecological character displacement. *Ecology*, 77, 510–520.
- Doebeli M. (2002). A model for the evolutionary dynamics of cross-feeding polymorphisms in microorganisms. *Popul. Ecol.*, 44, 59–70.
- Doebeli M. & Dieckmann U. (2000). Evolutionary branching and sympatric speciation caused by different types of ecological interactions. *Am. Nat.*, 156(Suppl.), S77–S101.
- Geritz S.A.H., Kisdi É., Meszéna G. & Metz J.A.J. (1998). Evolutionary singular strategies and the adaptive growth and branching of the evolutionary tree. *Evol. Ecol.*, 12, 35–57.
- Hartl D.L. & Clark A.G. (1997). *Principles of Population Genetics*, 3rd edn. Sinauer, Sunderland, MA.
- Helling R.B., Vargas C.N. & Adams J. (1987). Evolution of *Escherichia coli* during growth in a constant environment. *Genetics*, 116, 349–358.
- Kondrashov A.S. & Kondrashov F.A. (1999). Interactions among quantitative traits in the course of sympatric speciation. *Nature*, 400, 351–354.
- Lenski R.E. & Travisano M. (1994). Dynamics of adaptation and diversification: a 10,000 generation experiment with bacterial populations. *Proc. Natl Acad. Sci. U S A*, 91, 6808–6814.
- Levin B.R. (1988). Frequency-dependent selection in bacterial populations. *Phil. Trans. R. Soc. Lond. B*, 319, 459–472.
- MacLean R.C. & Bell G. (2003). Divergent evolution during an experimental adaptive radiation. *Proc. R. Soc. Lond. B*, 270, 1645–1650.
- MacLean R.C., Bell G. & Rainey P.B. (2004). The evolution of a pleiotropic fitness trade-off in *Pseudomonas fluorescens*. *Proc. Natl Acad. Sci. U S A*, 101, 8072–8077.
- Novick A. & Szilard L. (1950). Experiments with the chemostat on spontaneous mutations of bacteria. *Proc. Natl Acad. Sci. U S A*, 36, 708–720.
- Paquin C. & Adams J. (1983). Frequency of fixation of adaptive mutations is higher in evolving diploid than haploid yeast populations. *Nature*, 302, 495–500.
- Pfeiffer T. & Bonhoeffer S. (2004). Evolution of cross-feeding in microbial populations. *Am. Nat.*, 163, E126–E135.
- Porcher E., Tenaillon O. & Godelle B. (2001). From metabolism to polymorphism in bacterial populations: a theoretical study. *Evolution*, 55, 2181–2193.
- Rainey P.B. & Travisano M. (1998). Adaptive radiation in a heterogeneous environment. *Nature*, 394, 69–72.
- Rainey P.B., Buckling A., Kassen R. & Travisano M. (2000). The emergence and maintenance of diversity: insights from experimental bacterial populations. *TREE*, 15, 242–247.
- Riley M.A., Cooper V.S., Lenski R.E., Forney L.J. & Marsh T.L. (2001). Rapid phenotypic change and diversification of a soil bacterium during 1000 generations of experimental evolution. *Microbiology*, 147, 995–1006.
- Rosenzweig R.F., Sharp R.R., Treves D.S. & Adams J. (1994). Microbial evolution in a simple unstructured environment: genetic differentiation in *Escherichia coli*. *Genetics*, 137, 903–917.
- Rozen D.E. & Lenski R.E. (2000). Long-term experimental evolution in *Escherichia coli*. VIII. Dynamics of a balanced polymorphism. *Am. Nat.*, 155, 24–35.
- Slutner D. (1994). Experimental evidence that competition promotes divergence in an adaptive radiation. *Science*, 266, 798–801.
- Slutner D. (1995). Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology*, 76, 82–90.

- Schluter D. (2000a). Ecological character displacement in adaptive radiation. *Am. Nat.*, 156, S4–S16.
- Schluter D. (2000b). *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.
- Slatkin M. (1980). Ecological character displacement. *Ecology*, 61, 163–177.
- Spiers A.J., Kahn S.G., Bohannon J., Travisano M. & Rainey P.B. (2002). Adaptive divergence in experimental populations of *Pseudomonas fluorescens*. I. Genetic and phenotypic bases of wrinkly spreader fitness. *Genetics*, 161, 33–46.
- Spiers A.J., Bohannon J., Gehrig S.M. & Rainey P.B. (2003). Biofilm formation at the air-liquid interface by the *Pseudomonas fluorescens* SBW25 wrinkly spreader requires an acetylated form of cellulose. *Mol. Microbiol.*, 50, 15–27.
- Stewart F.M. & Levin B.R. (1973). Partitioning of resources and the outcome of interspecific competition: a model and some general considerations. *Am. Nat.*, 107, 171–198.
- Travisano M. & Rainey P.B. (2000). Studies of adaptive radiation using model microbial systems. *Am. Nat.*, 156, S35–S44.
- Treves D.S., Manning S. & Adams J. (1998). Repeated evolution of an acetate-crossfeeding polymorphism in long-term populations of *Escherichia coli*. *Mol. Biol. Evol.*, 15, 789–797.
- Turner P.E., Souza V. & Lenski R.E. (1996). Tests of ecological mechanisms promoting the stable coexistence of two bacterial genotypes. *Ecology*, 77, 2119–2129.

Editor, Masakado Kawata

Manuscript received 3 June 2004

First decision made 13 July 2004

Second decision made 13 September 2004

Manuscript accepted 23 September 2004